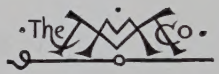
PROTOZOAN PARASITISM OF THE ALIMENTARY TRACT

Pathology, Diagnosis and Treatment



THE MACMILLAN COMPANY
NEW YORK · BOSTON · CHICAGO · DALLAS
ATLANTA · SAN FRANCISCO

MACMILLAN & CO., LIMITED
LONDON · BOMBAY · CALCUTTA
MELBOURNE

THE MACMILLAN COMPANY
OF CANADA, LIMITED
TORONTO

PROTOZOAN PARASITISM
OF THE
ALIMENTARY TRACT

Pathology, Diagnosis and Treatment

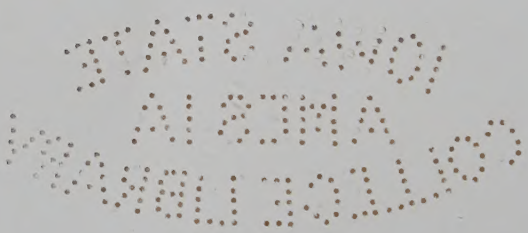
BY

KENNETH M. LYNCH, M.D.

PROFESSOR OF PATHOLOGY, MEDICAL COLLEGE OF THE
STATE OF SOUTH CAROLINA, CHARLESTON,
SOUTH CAROLINA

NEW YORK
THE MACMILLAN COMPANY
1930

Set up and electrotyped. Published November, 1930.



PRINTED IN THE UNITED STATES OF AMERICA

TO

JUANITA KIRK LYNCH

PREFACE

Eighteen years ago, when my interest in the protozoa of the alimentary tract of man was first aroused by my former chief and lamented teacher, the late Professor Allen J. Smith, of the University of Pennsylvania, there was but little actual knowledge concerning them in the possession of the medical profession.

In fact the whole subject of the relations existing between man and these lower animals, particularly in regard to the effect of the parasite upon the host, and, also, in reference to the relation of these protozoa of man to similar species in other animals, was in a state of confusion.

There had been, prior to that time, excellent work done in the subject, in fact, on the average, probably of better calibre than that of more recent studies, but it had not reached the stage of useful information available to the medical world.

Existing knowledge of protozoa in general was in the hands of a few men, biologists, or buried in scientific literature not available except by particular search; and the interest of the medical world in the matter had not been aroused. The conception of the protozoa of the alimentary tract, and of the nature of

their parasitism, in the minds of physicians in general was hazy indeed. Intestinal protozoa were parasites to be gotten in the tropics, the producers of amoebic and other tropical dysenteries. An amoeba was an amoeba and a flagellate was a "cercomonas"; most of the species now of familiar names were unknown to them, some were unrecognized by anyone.

Since that time, perhaps due largely to the interest incident to the world war and the care of large bodies of troops and other people, but primarily due to the persistent efforts of a few whose interest and knowledge in this field came into play under the conditions of war organization, the whole subject has come into general prominence. Much valuable information has been added by careful scientific investigators, but a great deal of undigested writing, especially by physicians who have not had particular regard for scientific methods, has been done. Consequently there exists considerable chaff with the grain, and the medical profession has been fairly confused by it.

During recent times there have been published several excellent books which cover the field in various fashions, such as Wenyon's *Protozoology,* Calkin's *Biology of the Protozoa,* Craig's *Parasitic Protozoa of Man, Human Protozoology* by Hegner and Taliaferro, Chandler's *Animal Parasites and Human Disease, The Intestinal Protozoa of Man,* by Dobell and O'Connor, and others, to which works the author refers those who desire biological information beyond

the scope of this book and bibliography not directly referred to here.

Most of the above mentioned works, excellent as they are, are difficult for any except those with a fair knowledge of the subject already and the most of them are of more service to biologists than to practitioners in preventive and curative medicine.

The confusion which still exists, almost as great as ever among those most practically concerned with the subject, has been the stimulus in attempting this book.

It has been planned as a monograph for students and practitioners of medicine and for those variously connected with medicine in special ways which bring them as responsible parties into this very common consideration in the diagnosis, prevention and treatment of disease in man.

It is not intended for protozoologists, save as a connecting link between their science and medicine. To biologists it will be an unconventional book. It is purposely so. Technical details which tend to confuse and so lose the interest of those to whom they are of no concern are largely left out of consideration.

To many medical practitioners it will probably be a radical book. In my several years of experience as a sort of a go-between in protozoology and medicine I have come to some very definite opinions as to certain practices in medicine which are difficult to justify. These conclusions may not be agreeable to certain practitioners.

To those physicians who diligently and honestly endeavor to do their full duty by their patients but who are widely and frankly confused as to what to do and when to do it when they are confronted with the finding of one or more of these organisms in an unwell person, I hope this book may be of some assistance.

To those who have become blinded by false report or faulty or inadequate observation, I hope it will bring serious reconsideration on the basis of the scientific method.

To those laboratorians connected with the medical profession as technical assistants I hope it may be of use in studying the several protozoa of this system which they are commonly called upon to identify.

The author desires to express his thanks to the Curator of the U. S. Army Medical Museum and to Major G. R. Callender for the use of certain photographs, which are noted in their titles as having come from the Museum, and to Dr. W. M. James, of Panama, and others whose material has been freely used. Appreciation is also expressed to Mr. J. M. Hicks, a student at this College, for his assistance in making the drawings for some of the illustrations, which drawings were not usually made from individual specimens of the various organisms but are mainly conceptions as set down for him by the author from study of actual specimens, usually a great number, assisted by the conceptions of other workers in this field of protozoology.

KENNETH M. LYNCH.

Charleston, S. C.

CONTENTS

INTRODUCTION

The problem of the status of the protozoa of the alimentary tract in relation to health and disease is of great medical and economic importance.

When we consider the great and world-wide prevalence of this class of parasites, the great number of people who are not in good health who carry one or more of species of these organisms, the great number of people who are being treated by physicians over extended periods of time with the finding of intestinal protozoa as the basis of treatment, the subject becomes of large proportions and of great consequence to the public of the world. This is a responsibility which the author would bring to the medical profession.

The second responsibility which belongs to them in the matter is this. The search for and identification of alimentary tract protozoa in the practice of medicine, as done in this country outside of recognized medical centers, is largely in the hands of unqualified people.

To many people who assume the responsibility for identifying protozoa in the feces and of playing some part in evaluating their relations to disease, that all-important body, the protozoan cyst, is an un-

recognized object. I believe that it is no exaggeration that many have not consciously observed a protozoan cyst, many are unable to differentiate the several species when they see them, and not a few are unable to differentiate that common organism, *Blastocystis,* from them.

In a great many instances the finding of an amoeba in the stool has furnished, per se, a diagnosis of intestinal amoebiasis. Correct identification of the amoeba is comparatively seldom done.

The finding of a free swimming form leads to a report of a flagellate, without much regard to the species. All are *Trichomonas* or *"Cercomonas"* to many medical laboratorians and practitioners.

These practices are to some extent a convenience. When the finding of protozoa fits with clinical ideas, or when nothing else is found as a basis for the illness, or, in the hands of many physicians, regardless of other conditions, the parasite is made the basis of therapeutic attack.

Many patients have been and are now under treatment for intestinal amoebiasis who have had no pathogenic amoeba. Many people have been and are now under treatment for "flagellosis" whose flagellates are not concerned in their illness or, literally, about the treatment.

Protozoologists as a rule are not pathologists; few pathologists and other physicians know much about protozoa. Therefore the errors common among protozoologists in evaluating disease when associated

with the presence of protozoa and among physicians in estimating the significance of the finding of protozoa in a sick person.

Particularly is the practicing physician prone to err when he is searching for the cause of a confusing disease and finds such an optically prominent thing as a protozoon in the stool. It appears as if it should be doing something harmful. The physician does not ordinarily become so concerned about the myriads of bacteria also present. They are not so prominent to the eye. This prominence of the relatively large protozoa is undoubtedly responsible for the difficulty which many have in passing them by. One may well wonder if more actual harm than good has not come from routine stool examination, as used up to the present time.

CHAPTER I

The Life of Protozoa

History and General Considerations. Anatomy and Physiology. Characteristics of the Cysts. Life History. Specificity of Parasite for Host. Resistance and Immunity of Hosts. Encystment. Reproduction.

HISTORY AND GENERAL CONSIDERATIONS OF PROTOZOA

The discovery that our universe is populated by living organisms of insufficient size to be seen by the unaided human eye belongs to Antoni van Leeuwenhoek (1632–1723), the great Dutch microscopist. He it was in 1675 who first saw, with the aid of a microscope of crude proportions as compared to the apparatus of the present day, minute animals which are called Protozoa. He studied them as free living forms, as parasites of lower animals, and as parasites of man, finding himself the host of one, which Dobell identifies as *Giardia (Lamblia) intestinalis.*

He thus became the discoverer of a world of life, unknown before that time and now unknown to the vast majority of people, the population of which far exceeds in number the visible animal population of the world.

Scattered far and wide over the earth, they live wherever they find sufficient moisture even for a short period of activity, lying dormant when their medium is no longer liquid. In their dormant phase, which we call cysts, they protect themselves by a capsule formed from their own secretion, in which they may survive drought conditions for variable lengths of time.

Warm wet regions are thus most favorable to them and the waters of such climates teem with their numbers. The sands of the desert, the dust of the earth and air, the ice of frozen regions, however, harbor them, waiting for favorable conditions for continued activity.

It is probably not to be supposed that the forms which we see today are the same as their ancestors, nor is it to be thought that they are the forbears of higher animals. Such is sometimes the conception of the ignorant concerning the meaning of evolution.

Undoubtedly the species which we know today as free living forms and as parasites upon higher animals and man have evolved from common ancestors. Adaptation to changed or new environment has produced division into species, races and strains, specifically suited to the environment in which they live. Thus the different species of one kind of protozoa living in freedom or as parasites in man and other animal hosts are close akin, at times difficult to distinguish from each other, and yet usually distinct

and not interchangeable from one kind of animal host to another.

The bodies of the active form of protozoa are naked and their locomotor apparatuses are but primitive. They are therefore largely dependent upon a liquid medium for their activities of movement and search for food and for protection from evaporation of the water of their own bodies. Some are dependent upon food in solution, also. Consequently the necessity of a fluid environment for them. Those which are not subject to periodic drying of their habitat and which do not need protection in passing from one host to another exist mainly in the naked active form and do not encyst.

Various temperatures of their fluid mediums are suitable to the various species. Activity of free living species goes on under considerable natural temperature variation. Activity in those parasitic in cold blooded animals has an optimum temperature corresponding to that of the host. In the protozoa of warm blooded animals warmth of the range of the host is the optimum state for activity and they usually cease motion and soon die upon cooling, even for a few degrees.

Naturally the naked forms are very susceptible to heat and the cysts are also killed at a definite and not high degree, as is known for some parasitic species. While cold readily kills the active protozoa of warm blooded animals, the free living and those of the cold blooded may survive and may even be active short of

freezing and the cysts of some may survive even that temperature.

Within the bodies of these minute beings occur all of the complex processes of life. They live and move and have a being. They move about purposefully, they ingest food, they inhale, they digest, they metabolize, they reproduce their kind. Under unfavorable conditions they sicken and die, under favorable environment their lives and their bodies are continued in their offspring; they are essentially immortal beings.

The construction and organization of their bodies is of the simplest, and at the same time complex. The secrets of life are concentrated here in a form which may be seen in toto, the different scenes enacted in periods of time so short as to be subject to continuous observation. Consequently many have thought and tried to determine what has remained hidden from the mind of man by study of the lives of protozoa.

ANATOMY AND PHYSIOLOGY

A protozoon is a minute animal, the organs and systems of which, with their specialized materials, are not separated into anatomical units called cells. The whole body bears physical resemblance to a single cell of the higher animals, in that in a general pattern it is composed of cytoplasm and nucleus, and in that sense is unicellular. Physiologically the pro-

tozoon is non-cellular. To designate a protozoon as a single cell or a unicellular animal seems inadequate or insufficient to some who are most familiar with them, but there seems to be no way to avoid the visual fact of a single celled body as long as the cell is the generally recognized unit of life and until we are in possession of deeper knowledge concerning the fundamental factors or units of life beyond the physical cell. Just as the fundamental unit of matter has changed in our conception with growing knowledge, so will our ideas of the unit of living matter change and perhaps there will come a time when cellular biology will be as inadequate as the molecular idea may be in physics.

Until then perhaps we should adhere to the conventional and speak of Protozoa as unicellular animals, as differentiated from Metazoa, or higher animals, in which the same or comparable vital organs or systems are organized as separate recognizable physical cells. It is common to think of the various systems of the protozoa as of low order, primitive. The size of their bodies, the accessibility of all parts to food, water and oxygen absorption, the ease with which waste materials may be cast out, the joining of the principals of somatic and germ life in one nucleus; all do away with the necessity for highly complex alimentary, digestive, circulatory, respiratory, excretory, reproductive, and nervous systems. Their organization is entirely adequate for their lives, in fact the advantage which they have over

larger and necessarily more complex animals is commonly manifest.

The bodies, then, of protozoa are organized into specialized systems corresponding to those which we know in higher animals, constructed of specialized materials but not of separate and specialized cells.

The outer part of the body is called the ectoplasm (outer substance). Here the protoplasm is of more uniform consistency, for a variable width in different forms. There is no separate surface covering or at least only a condensation which keeps the body intact.

From the surface are projected the organelles of locomotion and food gathering. Some of them possess for these purposes the ability to produce protrusions from their bodies, called pseudopodia (false foot). These are not permanent features but are projected on proper stimulus toward food materials or in the direction which the body is to travel. When a pseudopodium is used for gathering food it is extended to and wrapped around the article. This is taken directly into the protoplasm through a solution in the continuity of the rim, which immediately is healed.

When locomotion takes place by pseudopodia the projection of ectoplasm takes place in the desired direction and the inner substance, endoplasm, flows into it, much as a thick-walled flexible bladder of fluid may pass through a small aperture.

Pseudopodia are thus feet for moving and hands

for feeding. Their protrusion seems to be by external stimulus on the part of desirable food substances.

Thus there is a primitive sense system, corresponding to the sight, smell, taste, and touch organs of higher animals.

A protozoon possessing pseudopodia is known as an amoeba (change).

Some protozoa have, instead of pseudopodia, flagella (whip). A flagellum is a lash-like filament extended as a more or less permanent organelle from the surface of the body, the number and position of these processes varying with the species of flagellates, so named from this feature.

A flagellum is constructed of a filamentous core attached centrally to certain little bodies called blepharoplasts which are intimately connected with the nuclear apparatus, the central control organ, this core projected through the surface membrane or ectoplasm and carrying the covering with it. Flagella are moved in rythmic lash-like manner and serve as organs of locomotion and food gathering. They evidently have tactile sense. In food gathering they whip or brush particles into contact with the surface of the body, where they are then ingested. They are used as propellers in free swimming locomotion and protozoa possessing only this means of propulsion usually move about in a jerky manner. Smoother movement of some flagellates results from other swimming appendages or from a posteriorly

projected flagellum of spiral or screw-like movement.

Some flagellates have very pliable bodies, so that they may twist and turn and so force their way among solid materials in their liquid mediums by paths smaller than their bodies. Such pliable bodies give a darting action to the organisms. Others have very rigid bodies of unchanging contour. Some are twisted so that forward movement occurs in spirals.

Another specialized organelle of locomotion is the undulating membrane. This is a fin-like affair extending for various distances over the surface of the body. It has a stiffening rib, similar to the core of a flagellum and having a similar origin, which raises up a covering of ectoplasm to form a ruffle. The undulating or snake-like motion of this stiffening rib and ruffle-like covering furnishes a means of rapid motion. In a sluggish individual the slow undulation of this ruffle has been confused by some with pseudopod projection and has led to the report of an undulating amoeba (*Amoeba undulans*, Castellani).

Still another apparatus for locomotion and for feeding is a surface covering of cilia (eyelid). Cilia are comparatively short hair-like bristles which cover the bodies of the ciliates. They move in rythmic order like standing grain in a wind or the feet of a thousand-leg, creating currents to propel the organism or to sweep food materials into reach of the ingesting surface.

The different external appendages of protozoa,

then, serve the purpose of locomotion or of food gathering or both.

Protozoa are voracious feeders. They live to eat to multiply.

Some have no apparent mouthpiece but may engulf solid particles through a seemingly unbroken ectoplasm. Pseudopodia or ectoplasmic processes from any part of the body may wrap themselves around a food body and enclose it much as flowing water will surround a stone.

Some absorb foods in solution.

Some have stomata (mouth) which to most observers may appear more imaginary than real. They may be merely specialized surfaces through which food particles are taken when they are brushed against them by food gathering organelles. These mouthpieces are usually depressed at least.

Others have more definite stomata which may be readily seen and recognized as pores or orifices. Food materials are thrown into them by flagella or cilia.

Foods of various kinds, bacteria, yeasts, molds, other protozoa, starch granules, crystals, materials from the tissues of the host, liquids, etc., are thus ingested through the ectoplasm and are swallowed, so to speak, into the interior of the body, the endoplasm (inner substance).

Here they are brought into contact with the secretions, the digestive juices. Usually there is formed about one or more of these swallowed substances a cavity into which is poured the digestive fluid.

These cavities are commonly called vacuoles. They are essentially stomachs.

The gastro-intestinal system of the protozoa is therefore a remarkably adjustable one.

In these vacuoles the usable nutriment is prepared for absorption and metabolism. It is used or stored, as the need may be, the storing of reserve materials being apparently mainly a preparation for hibernation in the encysted phase, where sometimes multiplication takes place.

The waste from digestion, and probably from the completed metabolism, is expelled to the exterior. Excretion thus takes place, commonly by rupture of a vacuole containing waste material through some part of the ectoplasm, sometimes through a special or pore-like excretory aperture.

The smallness of the bodies of these animals and the accessibility of the protoplasm to the diffusion of gases enables them to do without special respiratory tracts and systems, necessary to larger animals. There are no recognizable structures directly concerned with respiration. Parasitic protozoa, living in the dark of the bodies of their hosts, possess no chromotophores (color bearer) by which oxygen may be separated from carbon dioxide of their mediums, such as some free living forms exposed to light have.

Internal metabolism by protozoa is carried on by more or less constant movement of the cytoplasm in currents.

The structure of the inner substance (endoplasm)

is generally alveolated, that is there exists a reticulated network frame structure, corresponding to the connective and framework tissues of higher animals. This network holds the fluid substances in order and allows the development of currents of interchange of substances of secretion, nutrition, and excretion, in other words a circulation adequate for the needs of the animal.

The center of life and its activities in protozoa is the nucleus. This body is located in the endoplasm, and corresponds in a general way to the nucleus of the cells of higher animals in its construction, essential character, and manner of participation in division of the organism. It has a limiting membrane enclosing a network of (linin) fibrils, within the meshes of which lies the fluid of the nucleus, and arranged on this network or clinging to the rim is the chromatin, the particularly essential nuclear substance. This chromatin is variously distributed in different forms and its arrangement is so typical in some that it is used as one basis of differentiating and identifying them. The common nuclear appearance of those with which we are here concerned is that with a definite rim, rounded in outline, with granules of chromatin of varying sizes clinging to the inner surface of the rim and also accumulated in or near the center in a mass called the karyosome. The linin network is more or less distinct and on its threads may appear chromatin granules.

The nucleus is necessary to the continued exist-

ence of a protozoon, it is the directing factor of its activities, it carries the hereditary characteristics of the species, and it naturally plays the essential rôle in division and the production of offspring. It is the directing force of somatic life and, at the same time, the germ of the offspring.

CHARACTERISTICS OF THE CYSTS

Each encysting species has its own peculiar cyst, the importance of which in identification of the parasite is not as widely recognized and attended to as it should be. Particularly is this the case in the two intestinal amoebae of man between which it is of so much importance in medicine to distinguish.

The shape and size of protozoan cysts are characteristic of the species, the contour being more nearly rounded than in the vegetative phase and the size usually reduced. The cyst wall or shell is of considerable thickness in some and of less definiteness in others. The body of the organism is condensed and usually motionless after the stage is fully developed. The cytoplasmic division into ectoplasm and endoplasm is not definite. The various organelles of the vegetative phase are indistinct or not seen. Undigested food bodies are absent but some contain stored materials, such as glycogen. Some contain "chromidial" bodies, in the shape of slivers, bars, or blobs, which take the nuclear stain. The nuclei of the cysts are characteristic of the species, in

form and number. In those which multiply within the cysts, the nucleus divides, by mitosis, and each daughter nucleus continues the division until the full number for the species is produced.

When the process of intracystic reproduction is incomplete the nuclei are usually large and hyperchromatic, when complete they are characteristic in size, shape and appearance.

Cysts in their natural state appear as glassy or hyaline bodies.

LIFE HISTORY

Protozoa either spend their lives as free-living animals in suitable environment, usually in water, or they live in connection with other animals. According to the nature of this association they may be commensals, which are dependent upon their association with their hosts but cause them no damage. Others live in symbiosis, where both of the parties to the association derive some benefit from it. Still others are parasitic upon their hosts, that is they cause some injury by utilization of materials needed by the host or by producing substances harmful to the host tissues. In this strict sense the protozoa here under consideration are either commensals or parasites and the debate has been as to which class the several kinds belong.

It has come to be the popular conception that "a living organism which, for the purpose of procuring food, takes up its abode temporarily or permanently,

on or within another living organism" is a parasite. There is something to be gained by adhering to this conception and, following Craig's example, I have taken this definition from Fantham, Stephens and Theobald (1916). In this sense all the organisms here considered are parasites of man and are so classed. Some of these parasites are pathogenic and some are apparently not.

As has been frequently called to attention the natural host of a parasite is one which has usually become adapted to it and which is ordinarily done comparatively little harm by it. Otherwise the parasite would have but little chance of survival, since it is dependent upon the opportunity of transmission to other hosts before its own death occurs with the death of its carrier. Consequently when severe damage is done the host by the parasite it is to be suspected that this is an unnatural association.

SPECIFICITY OF PARASITE FOR HOST

Further, it has come to general recognition that there is more or less rigid relationship between parasite and host, even to the point that most of them are able to survive only in their own particular hosts and in only one particular place in that host. These are very important points in considering the relation of protozoan parasitism in general to human medicine. Were this not the case, were the very great number of protozoa parasitic in other animals transmissible to

man, and were those of the several divisions of man's alimentary tract readily disseminated to other parts, the case would be tremendously complicated and much more important than it is. The popular conception that we receive our protozoan parasites from the animals with which we have surrounded ourselves is generally not true. Consequently, the matter of prevention comes to mean mainly the protection of man from contamination with the excreta of his own kind.

When, theoretically at least, we find a protozoon producing a definite disease as a constant or common result of its parasitism, it may be suspected that it is not a natural parasite of this host and search for its natural host should be made, since control of it would probably involve control in the natural host.

In naturally occurring infections there is ordinarily a state of balance between host and parasite. Occasionally this balance does not occur or is broken down.

RESISTANCE AND IMMUNITY OF HOSTS

Natural immunity, therefore, exists in most animals against the parasites of other species. This may be broken down at times. There is also natural immunity of some individuals to parasites of their own species, acquired immunity. Definite knowledge of the development of resistance to the parasites under question here, following their invasion, does not exist. It is indicated by Craig (1926), who reports

obtaining complement fixation between antigen made of cultures of *Endamoeba histolytica* and the blood of hosts of this parasite, by Wagener (1924), who found positive precipitation of extracts of scraped mucosa of amoebic colitis by blood serum of cats experimentally infected over a week with *Endamoeba histolytica* and negative controls in other cats, and by Wagener and Thomson (1924), who found that kittens previously infected with *Endamoeba histolytica* from chronic human amoebiasis were more difficult to infect with amoebae of more virulent strains than were normal animals of the same age and size. It is, perhaps, indicated by the experimental production by the author of encystment of *Trichomonas caviae* in the blood serum of its own individual host.

This occurred during the course of culture of *Trichomonas caviae* at 37.5°C. incubation in medium composed of one part of the animal's own fresh blood serum in nine parts of 0.9% sodium chloride solution, but did not occur in similar medium of other serum.

It is common knowledge that the number of parasitic protozoa to be found varies widely from time to time and that they may die out completely. This, however, may be due to alteration of the state or content of the medium in which they live and not to the development of "antibodies" by the host. The work of Hegner (1923), in attempting to control the number of flagellates in the intestine of the rat by a protein diet, indicates that the diet may be an im-

portant factor in determining the growth of such parasites after their introduction.

In this connection an observation of the writer in the production of experimental intestinal amoebiasis in the rat is, perhaps, worthy of note. Rats fed on stale white baker's bread exclusively were more readily infected than those on a more varied diet and the colitis found was more extensive and active.

The relation of the diet to the development of parasitism of these organisms and, further, to the effects produced upon the host by the parasite is a promising problem for investigation.

ENCYSTMENT

Most of the protozoa of the alimentary tract, as is the case in general with protozoa, are able to form about their bodies a resistant capsule, when conditions are not favorable for continued vegetative existence in their environment. In some this encystment is consonant with preparation for multiplication, so that when again ready for activity the animal emerges from the cyst in the form of its offspring.

Immediately prior to encystment there is a slowing up of the movement of the body and usually contraction to smaller size, extrusion of food materials from the body, and, in some, a slow revolvement of the body while the cyst is forming. This contracted stage is the "precystic" form.

Just what is responsible for the phenomenon of encystment is not known. In certain free living forms it occurs when the liquid medium of the organism is drying. In certain forms which inhabit the intestine it occurs in the lower parts, as the bowel content becomes more solid. In certain cultures of these organisms it has appeared to take place, in no regular fashion, as the medium became inspissated and concentrated. It has been recited above that the writer had the occasion to observe encystment of numbers of *Trichomonas caviae* in a medium of the serum from its own guinea pig host, whereas the change did not occur in similar medium from other guinea pigs.

Some of the cysts produced by the protozoa are purely for protective purposes, and only the one organism emerges from the cyst when conditions again are favorable. In others, while the cyst is protective, it is at the same time reproductive, and the organism may divide into two or more offspring, as the case may be, which may later emerge from the cyst.

In some protozoa encystment is a definite and regular part of the life cycle, essential to its continued life.

With most parasitic protozoa it is essential to the continuation of the species, in that it is the particular form in which they are able to survive exposure to the external world and the vicissitudes of transference to a new host.

In general the process of encystment has been evolved by the individual species as best suited to their needs, some for reproduction, some for protection in unfavorable environment and reproduction, some purely for protection, while some are not dependent upon such a change for either protective or reproduction purposes and do not encyst.

Neither is it definitely known what leads the enclosed organism to escape from its cyst when it reaches a favorable environment for its further activity. It has long been assumed that the digestive juices of the alimentary tract cause dissolution of the cyst and allow the imprisoned parasite to escape. It is possible, however, for these same organisms to escape and continue their activities without such digestive action on the exterior, as witness the cultures which may now be grown from cysts of some of these organisms. It seems, therefore, that their escape is due to their own "digestive" influences upon the shell which they have formed, some forms leaving a pore of more easily dissolved material through which they may escape.

In the writer's laboratory notes of 1922, before the recent reports of cultivation of various amoebae of man, occurs the record of attempts to grow man's intestinal amoebae in a serum salt solution mixture similar to some which have since been reported as suitable for such culture work. It was observed that after 24 hours incubation at 37°C. cysts were empty. Free amoebae were found but transplantation was

unsuccessful. In the light of the report of Yorke and Adams (1926), and that of Allen (1926), it is believed that observation of excystation of these amoebae was truly observed and that primary cultivation was successful. The illustration of the ruptured cysts by Yorke and Adams conforms essentially to those seen and drawn at that time. These writers believe, from their investigations, that excystation of *Endamoeba histolytica* requires only moisture and suitable temperature (37°C.), while Allen reports that in order for *Councilmania lafleuri* to excyst in culture the cysts must reach the proper phase and the medium must have necessary chemical and physical conditions, it occurring in their experiments in Ringer's solution with 0.01% dextrine but not in other media.

At any rate escape from the cysts takes place under conditions suitable for the free activity of the organism and it emerges as one or more, in the different forms.

REPRODUCTION

Although reproduction of protozoa may be sexually or asexually, the latter is the usual means in the species parasitic in the alimentary tract of man. Division occurs by binary fission as the means of multiplication in the vegetative phase, two identical bodies, but commonly smaller, resulting.

The nucleus first divides by mitosis and then the

cytoplasm is separated evenly for the two individuals.

In the case of multiplication within cysts, which occurs in some of these species, the mitotic division of nucleus and nuclei proceeds to the production of the number characteristically formed, although sometimes multiples of the usual number are seen, before division of cytoplasm takes place. In some the completion of the process does not occur until excystation, when the smaller offspring, corresponding to the number of nuclei, are liberated.

CHAPTER II

Dissemination and the Prevention of Infection

The protozoa of man's alimentary tract are commonly transmitted from the human carrier to a new host by contamination of materials taken into the mouth by secretions or excretions of the infected individual, in fact the commonness of these organisms in man is a concrete illustration of the real uncleanliness with which the human is still in contact, in spite of vaunted sanitary procedures. This has been particularly stressed by Boeck and Stiles (1923), and by Dobell and O'Connor (1921). The incidence of intestinal protozoa is a direct index of the swallowing of fecal matter.

Those which inhabit the mouth are probably transmitted by kissing and by the placing of things in the mouth which are contaminated with saliva from the infected person. Ready passage of the mouth protozoa from one person to another is indicated by their failure to encyst.

Of those of the intestine, the encysting species find this resistant phase necessary to survival of an interval between passage, with excreta, from their

hosts and the opportunity to be swallowed by another person. The failure of an intestinal species to encyst indicates that it is able to survive this period of external exposure in the vegetative form, and, also, to pass down the alimentary tract to its natural habitat unharmed by secretions above. This passage of the stomach by *Trichomonas* in active form we know takes place.

The non-encysting must necessarily be transferred in liquid or moist medium, consequently we may suspect the commonly ingested liquids or moist foods which are not heated in preparation. The observation (Lynch, 1926) that *Trichomonas* is a common parasite of warm regions and rural populations and uncommon in colder climates and urban and institutional inhabitants is in support of this belief.

It seems readily appreciable that the prevention of contamination of water and other liquids and fresh moist foods, or sufficient refrigeration, or, on the contrary, the heating of them to a degree sufficient to kill vegetative forms would bring about the elimination of unencysting intestinal protozoa as human parasites. Modern measures of sanitation, sewerage disposal, control of drinking water and milk, seem to be sufficient for the purpose and to be responsible for the low incidence of *Trichomonas* in people of such hygienic environment.

The species which are transmitted in the encysted state offer a different problem. Certain institutional surveys, notably by Thomas and Baumgartner

(1925), indicate that such hygienic measures are without avail in preventing the spread of these parasites and that close personal contact leads to a steady increase in incidence. Hand to mouth transmission and the food handler carrier are undoubtedly prominent means of spread of these parasites in places where there is proper disposal of human excreta and protection of water and food from fecal contamination. That being the case, protection against the carrier must be the means of prevention of such transmission.

In places where there is exposure and improper disposal of fecal matter, the transfer of cysts by flies and the contamination of food and drink by them are probably means of spreading these parasites, and prevention of such dissemination must mean the control of such conditions.

Flies may carry live protozoan cysts in their intestinal tracts, after ingesting them from human excreta, for probably several days, during which time they may contaminate food with them in their own excreta. They may also carry cysts upon their legs, although parasitic protozoa do not survive drying for any considerable period, even in cysts.

As for those which may be obtained from other animals there is one of importance which is apparently so spread, and there is another, the most important intestinal protozoon of man, which may possibly be spread in such a manner.

The former is *Balantidium coli,* a natural para-

site of swine, which is occasionally transmitted to man by swallowing materials contaminated with fecal matter from this animal. The means of prevention of such infection by those who may be exposed to it in handling hogs is evident.

The latter is *Endamoeba histolytica,* which has been shown by the writer and others to be capable of transmission to and parasitism in the common wild rat. If such should be the case in nature this is potentially a source of spread of the parasite, and the control of the rat, prevention of contamination of food by the excreta of it, and protection of human excreta from this animal, would be necessary to prevent such a manner of spread. It seems possible at the present writing that in regions where human excreta are exposed to rats which also have access to food of man, this natural means of transmission of this important parasite may occur. It is of sufficient importance to demand attention until proven or disproven, at any rate. More recently Kessel (1928) has found an amoeba, identified as *Endamoeba histolytica,* in naturally infected pigs in Peking, China, and has, also, brought about experimental infection of this animal by *Endamoeba histolytica* for a period of about six weeks. The pig is thus, also, accused of serving as a natural carrier of this amoeba and, if so, needs be guarded against in places where such an opportunity may be given it.

The transmission of these parasites, then, depends upon their natural habits of life, in relation to those

of man, and the prevention of it involves breaking the chain of sequence at some convenient or available point.

Thorough application of commonly recognized hygienic and sanitary procedures, personal and public, would probably suffice to control most if not all of these human infestations. Such a thorough application as to bring about their eradication is not to be anticipated, however, at the present or in the immediate future, particularly as regards personal hygiene and the control of the hand to mouth transmission, which is believed to be probably the most important means of spread of these parasites in modern community life.

It should be emphasized that the carrier furnishes the real problem in this division of preventive medicine. He it is who furnishes the transferable stage of the organism, not the person acutely ill of an infection which is being excreted in a non-transmissible stage of the organism. A patient with acute amoebic dysentery is of no danger to anyone else, the amoebae he passes are harmless. It is in the absence of diarrhoea that he becomes of danger to his fellow.

When it is called to mind that apparently about 10% of the population of any country harbors a pathogenic amoeba in transferable form and that probably from 30% to 50% of all people carry some intestinal protozoon, to say nothing of the organisms of the mouth, it is to realize how hopeless it is to contemplate any considerable reduction of

this class of human parasites in any given period of time.

Fortunately it seems unnecessary to even enter into such a consideration and it appears that the occasional cry of an alarmist is unwarranted. There is no indication of the post-war spread of intestinal amoebiasis which some professed to fear.

Particular conditions which must be considered and special measures which may be instituted in the prevention of the spread of these protozoa will be taken up under the consideration of the separate species.

TRANSMISSION OF ALIMENTARY TRACT PROTOZOA

1. By autoinfection. Transfer from one habitat to another in the same individual, in trophozoite stage.
   - A. *Trichomonas vaginalis*—to the mouth (?).
   - B. *Trichomonas hominis*—to the vagina (?).
   - C. *Trichomonas buccalis*—to the vagina (?).
2. By direct personal contact, in trophozoite stage.
   - A. *Endamoeba gingivalis.*
   - B. *Trichomonas buccalis.*
   - C. *Trichomonas vaginalis* (?).
3. By indirect means, in trophozoite stage.
   - A. *Trichomonas hominis.*
     (Contamination of food and drink by fecal matter).
   - B. *Chilomastix mesnili* (?).
     (Contamination of food and drink by fecal matter).
   - C. *Endamoeba gingivalis.*
     (Salivary contamination.)

D. *Trichomonas vaginalis.*
(Douche contamination ?).
E. *Trichomonas buccalis.*
(Salivary contamination.)

4. By indirect means, in encysted stage. Direct fecal contamination of food and drink and eating utensils or by means of flies.

A. *Endamoeba histolytica.*
B. *Endamoeba coli.*
C. *Endolimax nana.*
D. *Iodamoeba bütschlii.*
E. *Chilomastix mesnili.*
F. *Giardia intestinalis.*
G. *Embadomonas intestinalis.*
H. *Tricercomonas intestinalis.*
I. *Enteromonas hominis.*
J. *Balantidium coli.*
K. *Isospora belli.*
L. *Eimeria gubleri* (?).

5. Indirectly from man through the medium of another animal host, in encysted stage (?).

A. *Endamoeba histolytica.* (Monkey. Rat. Domestic pig.)
B. *Balantidium coli.* (Domestic pig.)

6. From other animals, themselves natural hosts of the parasite, in encysted stage. Direct fecal contamination of food and drink or of hands, or by mean of flies.

A. *Balantidium coli.* (Domestic pig.)
B. *Endamoeba histolytica.* (Rat?).

7. From other animals, themselves natural hosts of the parasite, in trophozoite stage. Salivary contamination of hands or objects or substances taken into the mouth.

A. *Endamoeba gingivalis* (?). (Dog. Monkey.)
B. *Trichomonas buccalis* (?). (Dog.)

CLASSIFICATION OF THE PROTOZOA OF THE ALIMENTARY TRACT OF MAN

PHYLUM	CLASS	ORDER	GENUS	SPECIES
Protozoa	Rhizopoda	Amoebida	Endamoeba	histolytica
				coli
				gingivalis
			Endolimax	nana
			Iodamoeba	bütschlii
			Dientamoeba	fragilis
	Mastigophora	Monadida	Trichomonas	hominis
				buccalis
			Chilomastix	mesnili
			Giardia	intestinalis
			Embadomonas	intestinalis
			Enteromonas	hominis
			Tricercomonas	intestinalis
	Sporozoa	Coccidiida	Eimeria	gubleri
			Isospora	belli
				hominis
	Ciliata	Heterotrichida	Balantidium	coli
				minutum
			Nyctotherus	baba

CHAPTER III

The Amoebae

Classification. General Considerations and Morphology. Methods of Examination for Amoebae.

CLASS: RHIZOPODA.
ORDER: AMOEBIDA.
GENUS: ENDAMOEBA.
SPECIES: *E. histolytica.*
E. coli.
E. gingivalis.
GENUS: ENDOLIMAX.
SPECIES: *E. nana.*
GENUS: IODAMOEBA.
SPECIES: *I. bütschlii.*
GENUS: DIENTAMOEBA.
SPECIES: *D. fragilis.*

THE AMOEBAE (AMOEBIDAE).

Of the order of Protozoa known as the Amoebidae, there are four well recognized genera parasitic in the alimentary tract of man, *Endamoeba, Endolimax, Iodamoeba, Dientamoeba,* and there are others not yet, I believe, sufficiently accepted, such as *Councilmania* and *Caudamoeba.* The amoebae are those

organisms which typically move and feed by the means of pseudopodia, that is by currents in their cytoplasm which may stream to a local part of the surface producing a finger-like projection, or may flow as a whole in one direction, leading to progressive locomotion.

The cytoplasm of the body shows division into a peripheral ectoplasm of apparently uniform consistency, and an inner endoplasm of more or less alveolated, vacuolated, or granular appearance. The ectoplasm is particularly specialized for the formation of pseudopodia, in other words conforms to and fulfills the purpose of the peripheral apparatus of all animals, *i.e.* locomotion, feeding, excretion, and maintaining contact with the external environment. In the endoplasm occur the nucleus, the digestive cavities, etc., in other words the interior apparatus of the animal body, the "vital organs" and systems.

This division into ectoplasm and endoplasm varies among the genera, between species, and with individuals.

The nucleus is usually single and varies among the species to give characteristics commonly depended upon for differentiation.

All but one of the species found in man are known to undergo encystment. The amoeba of the mouth, *Endamoeba gingivalis*, apparently does not encyst. It has no need of a phase for protection against external exposure since it naturally would be transmitted directly from person to person by contact,

e.g. in kissing, or through the intermediation of the common use by mouth of the same materials, such as in drinking, etc. On the other hand the amoebae which inhabit the intestine must live through the vicissitudes of passing out of the host, of external exposure for varying periods of time and in contact with various substances, and then through the digestive juices of the upper alimentary tract, particularly the stomach, before reaching their natural habitat again. Consequently encystment has been necessary for their survival. In the important ones, if not all of these amoebae, there is multiplication of nuclei to a definite number during encystment, preparatory to the liberation of its offspring.

Reproduction in the vegetative phase is by binary fission, with the production ordinarily of two individuals, this being the method of multiplication during their natural life in the intestine. It is doubted that the cysts hatch naturally until a new host is invaded since the cysts are formed as the organisms pass down the lower intestine to be excreted.

METHODS OF EXAMINATION FOR AMOEBAE

For study of the fresh active forms a portion of the fluid content of the natural habitat of the organisms is necessary. Depending on the consistency of this medium it may be necessary to mix it with physiological saline solution. The material should be placed on a microscopic slide in thin enough state to

be clear to microscopic vision under a cover glass. The temperature of the medium should be near that of the body. In ordinary summer weather they will usually live and remain active for several hours. In the winter the material may be kept in the ordinary bacteriological incubator, or otherwise at about body temperature, in which case the amoebae will usually be active enough during the time of the examination, unless the room is cold. Tough mucus and thick or heavy particles of solid matter should be avoided. Ordinarily it is sufficient to take a bit of the material to be examined on the end of a match stick or wooden applicator stick and mix it uniformly with a drop of saline solution on the slide, after warming it to about body temperature. In case of a thin watery stool or of a mucinous stool it may not be necessary to use saline solution.

The active amoebae will be found after a short time to emerge from the heavier particles of the preparation and to cross the streams of liquid. After they have been located by the use of the ordinary low dry lens, they may be studied with the high dry objective, the oil-immersion being of little service beyond this. Dark field examination is striking but reveals nothing in addition. The points which may be determined from this examination are the size, the visibility of the nucleus, the character of the pseudopodia, the nature and activity of locomotion, the contrast of ectoplasm and endoplasm, the kind of food particles in the vacuoles of the endoplasm.

It is well to have such fresh liquid material for the determination of the presence of amoebae. It is not by any means always possible to identify them under these conditions. Frequent errors are made in diagnosis by depending upon this preliminary part of the examination. When amoebae are found it may be possible to form a good idea as to their identity, frequently it is impossible, always it is advisable to simply recognize the presence of an amoeba and, if no cysts are to be found, to wait until they may be before making a positive identification. This does not mean that it is necessary to wait for the cysts of *Endamoeba histolytica* before a diagnosis of intestinal amoebiasis is made. The character of the amoeba, its common red blood corpuscle inclusion, and the clinical nature of the case, particularly the nature of the stool, practically always allows a diagnosis of amoebic dysentery at once and with positiveness.

In chronic intestinal amoebiasis without dysentery discovery of the cysts is practical and should be done for a positive diagnosis.

For better study of the nucleus and composition of the body, food particles, etc., stained specimens are necessary. The best stained preparations are to be secured by the iron-haematoxylon methods about as follows:

1. Make thin spreads on slides (cover glass preparations are less easily handled) and before they can dry fix them in warm "Schaudinn's solution" (2 parts saturated solution of bichloride of mercury to 1 part

of absolute alcohol, to each 100 cc. of which mixture is added 5 cc. of glacial acetic acid) for 10–30 minutes.

2. Rinse in 50% alcohol.

3. Remove the remainder of the corrosive sublimate by placing in a weak alcoholic solution of iodine for about 15 minutes.

4. Pass through alcohols, 70%, 80%, and 90%, and wash in distilled water.

5. Mordant in 4% iron-ammonia-alum overnight.

6. Rinse in distilled water and from weak to strong grades of alcohol.

7. Stain in 1% alcoholic haematoxylon until the films are well blackened.

8. Rinse the excess of stain in water and differentiate in 2% iron-ammonia-alum. This is the step requiring most skill and attention. It can be mastered only by experience.

9. When decolorized to the correct point, usually just beyond the point when the color comes from the film into the differentiating fluid as the slide is dipped up and down, rinse in tap water, pass through graded alcohols and into carbol-xylol (or similar clearing agent), xylol, and mount in balsam.

Do not allow the film to dry at any stage.

This staining procedure is not easy and many poor specimens to each good one will be obtained, particularly until the technique is mastered and is being fairly constantly practiced.

Shorter methods of staining may be used, such as Mayer's "Haemalum," but the details are not as well presented as in the iron-haematoxylon.

Mayer's Haemalum may be made by dissolving 1 gram of haematoxylon crystals in 1000 cc. of distilled water and adding 50 grams of potash alum and 0.2 gram of sodium iodate.

The fixed and washed slide preparations from the fourth step in the technique given above for the iron-haematoxylon method should be placed in this stain for 15–20 minutes. The slides are then washed in tap water until blue in color, with the water running or being frequently changed. The preparations are dehydrated, cleared and mounted as above.

The iron-haematoxylon staining is best for careful study of nuclear and other structural detail. Mayer's Haemalum is much shorter and less difficult and is sufficient for identifying the organisms.

Study of fresh unstained materials for the active amoebae is only a preliminary step in the identification of the organisms. In the hands of an experienced protozoologist it may be conclusive in the majority of instances but it is reasonably certain that even the expert will err at times. Study of preparations stained by the iron-haematoxylon method will usually lead to accurate identification in the vegetative phase by the expert protozoologist. These measures, however, are not to be depended upon in less expert hands. The amoebae are frequently in such active form as to render unreliable an opinion as

to the species by any except the most experienced and at times by them, while this painstaking method of staining is usually not attended by striking success except by one constantly practiced in it.

It is considered a good practice in medical diagnosis to have some routine procedure like the following:

Unless there is diarrhoea, administer a saline cathartic to produce a liquid stool. Oil renders a specimen unfit for examination, both fresh and stained. Examine immediately, *i.e.* while fresh and without allowing the stool to cool, portions from different parts of the stool, as described above. Use a mechanical stage so as to be certain of covering the whole preparation. There has been much said about the frequency with which amoebae are not to be found in the stool of an infected person and it is well known that the fifth or sixth examination of a stool may show an amoeba for the first time. However, a fresh liquid stool, produced and examined under the above conditions, will seldom fail to reveal any existing infection. If two or three repetitions of the procedure fail to show any protozoon it may be ordinarily accepted that there is no infection.

So much emphasis has been placed upon the necessity for a number of stool examinations before a negative may be declared that it is often considered by practicing physicians that stool examination for protozoa is an impractical procedure.

For routine practice one or two repetitions of a negative finding, after two or three days, under strict

conditions as above may reasonably exclude protozoal infection.

The finding of an active amoeba by such examination is merely the first step. It must be now identified. If the case is one of clinical active intestinal amoebiasis, especially with blood stained mucous stool, and an amoeba is found, of active progressive motility, without bacteria and other solid bodies, such as yeasts or crystals, but perhaps containing red blood corpuscles, the presumption is that it is *Endamoeba histolytica*. This is allowable because it is advisable to get the individual on proper treatment and the diarrhoea may prevent the appearance of cysts for an indefinite period. In such a case the cysts may be hunted when the stool begins to form.

In other cases search must now be instituted for the cyst, which may or may not be found in a purged stool. If it is not, a formed stool must be obtained.

Study of amoebic cysts for diagnostic purposes in medical practice amounts to looking for that of *Endamoeba histolytica* to the exclusion of all others. Amoeba cysts in the stool are rounded, glassy or hyaline bodies, easily overlooked, sometimes very numerous, sometimes not.

Search for them is facilitated by mixing the particle of stool on a slide with an iodine solution, such as Gram's iodine (iodine 1 part, KI 2 parts, water 100 parts). This stains them various shades of brown

and makes them more conspicuous. It also allows the nuclei to become visible as colorless rings against the yellow-brown background.

When a suspicious body is located with the low-power lens, the higher dry magnification will usually reveal its structure.

If the cysts found show four round nuclei with tiny central karyosome it is *Endamoeba histolytica.* The main confusion is with the cyst of *Endamoeba coli;* it has eight nuclei. This simple mathematical proposition, so difficult to get across to medical laboratorians, is the most important thing in the diagnosis of amoebiasis, it is the sole conclusive point in the chronic case, practically considered.

Of course, there may be a mixed infection and one should not cease search for this cyst until satisfied of its absence, regardless of what else may be found.

In addition to *Endamoeba coli* much confusion arises from that ubiquitous stool inhabitant, *Blastocystis.* A little experience, however, will lead one to pass this by as he would foreign particles. Because of this common confusion consideration of *Blastocystis* in an appropriate place is given in this book, although it is not a protozoon. Suffice it to say here that it is commonly very numerous in a liquid stool, is very common in any, is of widely variable size, rounded, ovoid, or elongate. It usually has a transparent rim sharply defined from the main body, which is hyaline, stains brown with the iodine and contains no nuclei like those of the amoebae. Study

of illustrations of this body should lead immediately to its recognition.

For certainty in eliminating the possibility of *Endamoeba* in the stool, more than these fresh immediately examined preparations is necessary. Permanent stained specimens, by the haematoxylon methods previously described, are recommended by James (1927) and other experienced workers, on the contention that amoebae may frequently be found in these preparations when not in others and that they may be more accurately identified.

Consequently, the writer desires to recommend such a measure when *Endamoeba histolytica* has not been found in a suspicious case or when it has been identified by any measure except actual counting of characteristic nuclei in the cysts. It is freely confessed, however, that his ideas do not follow this far, in consideration of what is simple and therefore practical and likely to be done in medical practice outside of centers where the ultimate may be consistently followed.

It is emphasized that only a few are considered competent to identify anything but the cyst, and that familiarity with this stage should be stimulated with medical laboratorians.

Neither are cultures to be relied upon in any but expert hands, although some have recommended them for diagnosis in amoebic and other protozoon intestinal infections. With the experienced and expert cultures may pick up an infection otherwise

missed. With others they are unreliable and will undoubtedly lead to errors.

Methods of concentration of the cysts in the stool so that they may be more readily found are strongly recommended by some. For this purpose the procedure of Yorke and Adams (1926) is recommended.

About 10 grams of the feces, thoroughly mixed with 200 cc. of distilled water, is allowed to stand for 20–30 minutes. The supernatent fluid is poured off and distilled water to 500 cc. added. After standing overnight in the ice box the supernatent fluid of this is decanted and the sediment saved. This is washed once with distilled water and centrifugalized at about 2000 revolutions per minute for two minutes. The sediment is suspended in a solution of sucrose of about 1.080 specific gravity in about the proportion of 2 cc. of sediment to 14 cc. of sucrose solution. This mixture is next centrifugalized at 3000 revolutions per minute for 3 minutes. The supernatent solution, which contains the cysts, is concentrated by dilution with three volumes of distilled water and centrifugalizing at 2000 revolutions per minute for 2 minutes. A small portion of the sediment is examined for the cysts in microscopic preparation with iodine solution.

Without the intention of detracting from the absolute value of permanent staining, concentration methods and cultures, and recognizing their full value where they may be used in a competent way, particularly for uncovering amoebic infections when the parasites are sparse, it is the desire of the writer

at this time to encourage the performance of a simple procedure which will suffice in a practical way in the practice of medicine and will at least vastly improve upon present practices and conditions.

For this purpose it is desired to summarize the following procedure for a routine:

1. When a formed stool is up for examination, as it commonly is, make slide preparations with salt solution from various portions and look for motile and encysted forms. If motile forms are found search for the cysts. Search for cysts with slide preparations in the iodine solution. When found count the nuclei by fine adjustment manipulation of the high dry lens. If there are four characteristic nuclei report it *Endamoeba histolytica.* If only active forms are present, so report them and ask for continued stool examination until the cysts are found.

2. When a diarrhoeal stool is up for examination, look also for the cysts. Usually they will not be found. If there is an actively traveling amoeba which does not contain large vacuoles with bacteria and other foreign objects, but some contain red blood corpuscles, with clear ectoplasm and pseudopodia, and granular endoplasm, report it as a motile amoeba, possibly *Endamoeba histolytica,* and ask for continued stool examination for cysts.

As a routine practice, except in case of diarrhoea, ask for stools produced by saline purgative, not oil, and search for motile forms and cysts. If only motile

forms are seen, report it so and ask for continued examination for cysts.

3. When not positive of the identification of an amoeba, either motile or cyst, or when none is found where it is judged it should be, make slide preparations, at least twelve, according to the instructions of steps one, two, three, and up to the 90–95% alcohol of step four in the iron-haematoxylon staining technique previously given. Then seal in a container of alcohol and send to a recognized protozoologist for completion of the stain and search for and identification of the amoeba.

Where facilities and personnel of medical laboratories is sufficient the more elaborate procedures should be appropriately carried through.

CHAPTER IV

The Amoebae (Continued)

Endamoeba histolytica. Prevalence, Distribution and Transmission. Experimental Information. Cultivation. Habitat and Effects. Chronic Intestinal Amoebiasis. Acute Amoebic Colitis (Amoebic Dysentery). The Stools of Dysentery. Treatment.

ENDAMOEBA HISTOLYTICA.

This true parasite, of known pathogenic activity, is the most important of the protozoa of the alimentary tract. Discovered by Lösch (1875), it has been extensively studied by numerous observers and our information about it is probably more definite than with any of the other alimentary tract protozoa.

In active stage it is usually of rather characteristic appearance. Rounded up it measures 20 to 30 microns in diameter. When motile it travels rather actively and steadily, the ectoplasm in pseudopodia, at the forefront, the endoplasm with food bodies, if present, following, the flow of the whole organism being somewhat like that of a drop of liquid on an incline to which it adheres somewhat. The ectoplasm is comparatively clear, in pseudopodia when

motile, as a rim when rounded. The endoplasm is granular but not coarsely so as in some of the other amoebae. In this part of the body may be seen red blood corpuscles from the host, in amoebic dysentery. It does not contain bacteria and other solid food bod-

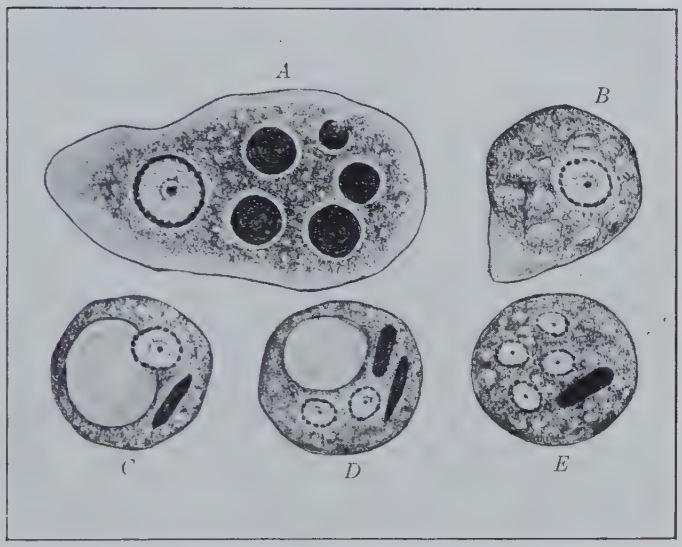


FIG. 1.—*Endamoeba histolytica.*

A, trophozoite with ingested red blood corpuscles. *B*, precystic stage. *C*, *D* and *E*, cysts with one, two and four nuclei. Drawings of specimens stained with iron-haematoxylon. x 2000.

ies in this natural state, although it may in culture, since these objects do not ordinarily form part of the food of this amoeba. The nucleus is invisible in the unstained specimen.

Stained by the iron-haematoxylon method, with light eosin counterstain, the ectoplasm is very light

pink or practically unstained, the outlines of the organism being somewhat indistinct at times. The endoplasm is a deeper pink, granular, and if red blood corpuscles are present they are rounded bodies retaining the black stain in proportion to the differentiation. The nucleus is here seen in characteristic form, from 4 to 7 microns in diameter, depending on the size of the amoeba. It is round, has a delicate but definite rim and a linin network, more imaginary than distinct as a common thing. Small chromatin granules are evenly distributed on the inner aspect of the rim and the karyosome is a minute chromatin body, centrally placed and surrounded by a "halo," which is more or less distinct. Such a stained amoeba may be readily identified.

The cysts vary in size according to the race, the different races averaging 6.6, 8.3, 11.6, 13.3, and 15 microns, as measured by Dobell and Jepps (1917–1918). They are round glassy or hyaline bodies in the fresh unstained state. With careful adjustment of the lighting the nuclei may be brought out as delicate refractive rings, four in number in the developed cyst. The chromidial bodies, characteristic of the species, may also be made out in such specimens by careful adjustment of the light. The main differential measure, upon which cysts of this amoeba may be identified immediately, without staining, is that of counting the characteristic nuclei.

In the iron-haematoxylon preparation these cysts are well stained, the cytoplasm light, the nuclei of

the typical form described in the trophozoite, the chromidial bodies black or dark brown bars or rods with blunt or tapering ends.

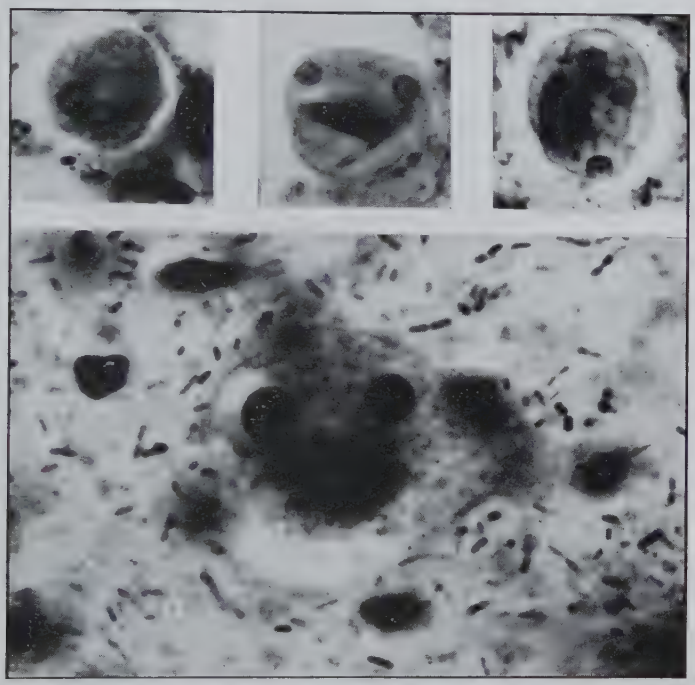


FIG. 2.—*Endamoeba histolytica.*

A, precystic stage. *B*, cyst with two nuclei and chromatoidal bar. *C*, cyst with four nuclei and chromatoidal bar. *D*, trophozoite with ingested blood corpuscles. Photomicrographs of specimens from man. *A*, *B* and *C* from chronic intestinal amoebiasis, *D* from amoebic dysentery, stained by iron-haematoxylon. x 1500.

PREVALENCE, DISTRIBUTION AND TRANSMISSION

Endamoeba histolytica, once thought to be of importance only in the tropics, is of world-wide distribu-

tion. In temperate or colder regions its incidence is usually less, but it has been found wherever search has been made for it. The reported incidence in different surveys varies widely, from around 1% to 20% and more, the variation in reliable studies apparently depending largely on the nature of the examined people, as to the sanitation of their environment and whether they are well or subjects of intestinal disease. The greater incidence is naturally where the protection of man from the excreta of his kind is poorest, this usually being the case in tropical and subtropical regions. In institutional life, with its close personal contact, it is likely to be comparatively high. In rural districts without modern facilities for sewage disposal it is also apt to be high. In modern communities with regulated sewage disposal, care of milk and other food, and water, it is low. Through a country like the United States it will probably average less than 10%. In the experience of the writer the incidence in the population of the modern city and town is nearer 3%. The food handler carrier and close personal contact with infected persons are undoubtedly the main means of spread of the parasite among the inhabitants of districts of good sanitary facilities. Prolific spread and the production of epidemics of disease by it are not at all likely, although Craig (1926) shows that epidemics in special groups, such as troops, exposed to massive infection under certain conditions may occur. The concern of some about the danger to the people of this country from

returning soldiers after the last war did not seem justifiable and has apparently not proven warranted.

Since the cyst is the only infective stage of the parasite, prevention of spread of it depends upon preventing this body from reaching the mouth of man. The control of flies and the protection of excreta and food from them is important. In regions where human excreta are used as fertilizer uncooked vegetables are dangerous. Public food handlers of all sorts may act as carriers and well regulated protection should involve examination of them for this organism. It is the apparently healthy carrier, not the person with amoebic dysentery, who is the dangerous factor in transmitting *Endamoeba histolytica*. Protection against this amoeba involves such sanitary practices as are used against typhoid infection, including consideration of the carrier. Walker (1911) names those who have recovered from amebic dysentery but still harbor the organism "convalescent carriers" and the person who has the parasite without having suffered from the acute disease the "contact carrier."

As previously referred to, the writer (1915) discovered a focus of natural infection of wild rats with an amoeba indistinguishable from *Endamoeba histolytica* in a house occupied by negroes, where a cellar was used as a "privy." From this habitation at least two cases of amoebic dysentery were traced. Brug (1918) found natural infection of two wild rats in Java. Chiang (1925) also discovered in laboratory

rats a natural intestinal infection with an amoeba agreeing very closely in its morphology with *Endamoeba histolytica* of human origin. These three, as well as Kessel (1923), also infected rats by feeding human feces containing the cysts. The rat is an omniverous animal and will eat human feces even when other food is available. In many places this animal has access to human excreta and opportunity to contaminate human food and eating utensils with its own excreta. These opportunities are uncommon under modern housing and sanitary conditions, but there are many places where they are not uncommon and these are the places where intestinal amoebiasis is usually most common and important. Until studies are made in such regions and it is shown that the rat does not transmit the infection in nature, this animal must be under suspicion as a possible carrier.

Under similar suspicion is the domestic pig, since Kessel (1928) has reported that this animal may naturally and experimentally harbor this amoeba for about six weeks. In some quarters the pig is in close contact with human beings and is allowed access to human excrement.

Using the culture method to prove the viability of cysts, Yorke and Adams (1926) found that *Endamoeba histolytica* cysts commence to die fairly rapidly in feces at laboratory temperature (16–20°C.) for three or four days and that all died within ten days. This was also the case at 0°C. They found, also, that the cysts survive a temperature of 45°C. for

thirty minutes but are killed within five minutes at 50°C. Ordinary chlorination of water, such as is commonly used in public water purification was without effect upon them but they were fairly sensitive to bichloride of mercury (1 to 2500), formaldehyde (0.5%), carbolic acid (1.0%) and lysol (1.0%). Boeck (1921), using eosin absorption as a test of the viability of cysts, indicated that this phase of *Endamoeba histolytica* survives for months in plain water.

Whether either of these methods, and which the better, suffices to determine when protozoan cysts are no longer able to hatch living organisms in the intestine remains to be conclusively shown.

Stools, water, and other liquids and moist materials containing *Endamoeba histolytica* cysts may be considered infectious for some time at least. Contaminated water is not freed of its infectiousness during this time by ordinary chlorination. The heating of foods which may have been exposed to contamination to 55°–60°C., the pasteurization of milk, similar heating or boiling of drinking water, should control the dissemination of the infection, where proper sewage disposal is not done, except the hand-to-mouth carrier transmission. When proper sewage disposal is not available, fecal matter should be disinfected by an agent of known amoebicidal properties (Craig [1926] recommends mixing the stool in cresol 1 to 200 for 15 minutes) and fly control and screening should be carefully exercised. Where human excreta is used

for fertilizing garden stuff, fresh uncooked vegetables, especially those kept moist, should not be eaten. Possibly refrigeration of such articles for a period of time, yet to be positively determined, sufficient for the cysts to die, may render them safe for consumption.

EXPERIMENTAL INFORMATION

Experimental infection of other animals with *Endamoeba histolytica* from man has been done on numerous occasions and has proven of great value in determining the manner of occurrence of the infection and the nature of the disease produced by it.

The most commonly used animal is the cat, kittens being susceptible to the infection on injection of material containing the amoeba by rectum, less so on feeding the cysts. The lesions produced in such a manner are similar to those of amoebic dysentery in man, including the occasional occurrence of amoebic abscess of the liver. Since Hlava (1887) first produced this experimental infection it has been frequently done, the early experimenters being Kartulis (1891), Kovacs (1892), Quincke and Roos (1893), and Kruse and Pasquale (1894).

Several workers, including Losch (1875), Hlava (1887), Kruse and Pasquale (1894), and others, have infected dogs.

Guinea pigs have been experimentally infected by Baetjer and Sellards (1914) and Chatton (1917).

Thompson (1926) produced experimental infection of rabbits.

Lynch (1915 and 1928), Brug (1919), Kessel (1923), and Chiang (1925), have brought about experimental infection of rats by feeding the cysts.

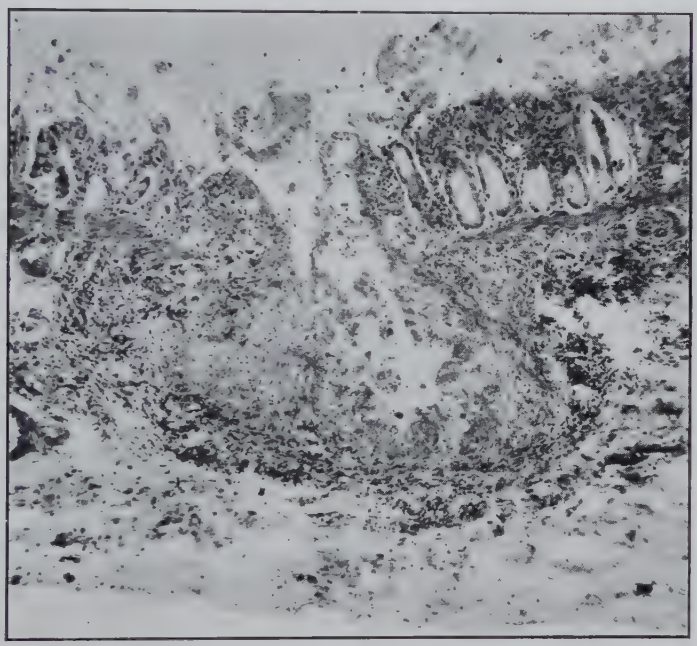


Fig. 3.—Experimental Amoebic Ulcer of the Colon of a Rat.

While the disease produced in kittens is similar to amebic dysentery in man, it is commonly very acute and it is practically always fatal. There is not the non-dysenteric phase as in man, consequently there is no encystment of the amoeba and this animal

cannot be a transmitter of the infection either naturally or experimentally. The amoebae can be transferred from cat to cat only by rectal injection of the living trophozoites.

The experimental disease in the guinea pig has been the formation of tumor-like growth of the infected wall of the caecum.

In rats there is the acute colitis corresponding to amoebic dysentery, as well as the development of the non-dysenteric carrier, with minute ulcers of the mucosa of the colon or without any discovered lesion.

CULTIVATION

There have been a great many unsuccessful attempts to obtain growth of *Endamoeba histolytica* outside of the animal intestine. Until very recently a good deal of confusion existed as to whether or not it had been done. Certainly a number of reports of its cultivation are erroneous, the amoebae grown being free-living forms accidentally encountered in the material used.

In the light of subsequent successes it appears that the first cultivation of *Endamoeba histolytica*, or any true parasitic amoeba, was done by Cutler (1918). Several workers failed to repeat Cutler's success, notably Dobell (1919), and Wenyon, and, consequently, it was taken as other reports had been.

Following the artificial cultivation of *Endamoeba barreti* by Barret and Smith (1923), comes the re-

peatedly confirmed and elaborated work of Boeck and Drbohlav (1925), and *Endamoeba histolytica* is now subject to laboratory growth and study in artificial media, although not in pure culture free from bacteria.

The medium used by Boeck and Drbohlav, as improved by Drbohlav, is prepared as follows:

Take four eggs, wash and brush with alcohol, break into a sterile flask containing glass beads, add 50 cc. of Locke's physiological solution and shake until the mixture is well broken up. Slant in test tubes to the length of 1 to 1½ inches and coagulate by heat in an inspissator at 70°C. Transfer to the autoclave and sterilize for 20 minutes at 15 pounds pressure. Cover the solidified egg to a depth of about 1 cm. above the slant with a mixture of 1 part of sterile inactivated human blood serum in 8 parts of sterile Locke's solution. Instead of the blood serum mixture 1% solution of crystallized egg albumin in Locke's solution may be used. Either of these mixtures may be sterilized by passing through the Berkefeld filter. Their sterility should be tested by inoculation and incubation of bouillon tubes or agar plates.

The Locke solution is made as follows:

Distilled water, 1000 cc.; NaCl, 9.0 gms.; $CaCl_2$, 0.2 gms.; KCl, 0.4 gms.; $NaHCO_3$, 0.2 gms.; Glucose, 2.5 gms. Sterilized either in the Arnold or autoclave.

The amoebae do not penetrate the solid medium but occur in the sediment of the lower part of the

surface fluid. The cultures should be incubated at 37°C. and transplanted on the second day, when the amoebae are most numerous.

Kofoid and Wagener (1926) substituted for the human blood serum in this medium rabbit, rat, cat, and guinea pig blood. Their most satisfactory substitution was defibrinated rabbit blood, used 0.5% in Locke's solution.

Craig (1926) succeeded in growing *Endamoeba histolytica* in seven parts of 0.85% sodium chloride solution and one part of inactivated human blood serum.

As Smith and Barret (1928) call to attention, Craig's medium is a variation of a mixture of blood serum which they, or one of them (Barret, 1921; Barret and Yarbrough, 1921; Barret and Smith, 1924; Barret and Smith 1926) have used to cultivate *Blastocystis hominis, Balantidium coli, Endamoeba barreti,* and *Endamoeba ranarum.* They suggest that variations of the strength of this simple medium may prove satisfactory for cultivation of a large range of parasitic protozoa.

The author has used it for more than ten years, along with similar mixtures of pleural and abdominal cavity fluids for cultivating several flagellates, as well as *Blastocystis.* Some of this work has been published previously and will be referred to under appropriate subjects; the first was not reported.

There appear to be unsolved difficulties in all of these methods of cultivation. In the writer's labora-

tory notes of 1922 occur observations of apparent multiplication of *Endamoeba histolytica* and *Endamoeba coli* in 1 to 5 serum mixture with 0.9% sodium chloride solution, incubated, at 37°C. This cultivation was ungovernable, however. It was a hit or miss procedure. Subculture was, also, ungovernable. The organisms multiplied for two or three days and then gradually disappeared by the fourth or fifth. The undeterminable and ungovernable factors were thought to be the bacterial content of the cultures and the changing reaction of the media.

It has appeared to the writer in working with the various described media for cultivation of alimentary tract protozoa that their reliability is by no means comparable to that of bacterial cultures. The content and reaction of the media is controllable only primarily.

The subsequent changes in the media must be variable and uncontrollable, as are the contaminating bacteria in them. Apparently identical media will grow them at times and will fail at others. Until the bacterial growth can be eliminated or controlled, cultivation of these protozoa will not be satisfactory and the benefits from such procedures will not be fully realized, although in the hands of some (Craig, 1928, and Craig and St. John, 1927) cultivation of *Endamoeba histolytica* has been reliable enough for recommendation as a measure of diagnosis of the infection.

HABITAT AND EFFECTS

When the cysts of *Endamoeba histolytica* are swallowed by man they pass unharmed to the intestine, where the cyst is dissolved or ruptured and the trophozoite liberated. The organism is probably liberated as a four nucleated amoeba and then completes the division. The daughter amoebae pass down to the large intestine, the natural habitat. Growing to mature size they become seated largely in the portions of this tract in which the content accumulates and remains longest, notably the caecum and the descending colon, although the whole of the colon may be involved in the infection, especially in amoebic dysentery, and there may be ulcers on and above the ileo-caecal valve.

Depending upon unknown factors, involving the capabilities of the parasite for invasion of the tissue as balanced against the resistance of the host to it, the results of the infection may be chronic intestinal amoebiasis or clinical amoebic dysentery, the former more commonly.

The manner of penetration and invasion of the wall of the intestine is subject to some debate, as to whether the amoebae pass through an intact mucosa between living epithelial cells or produce destruction of epithelium and a gateway for their admission. For many years it was believed by some that they gained entrance through lesions of other primary production. This is evidently not essential and they

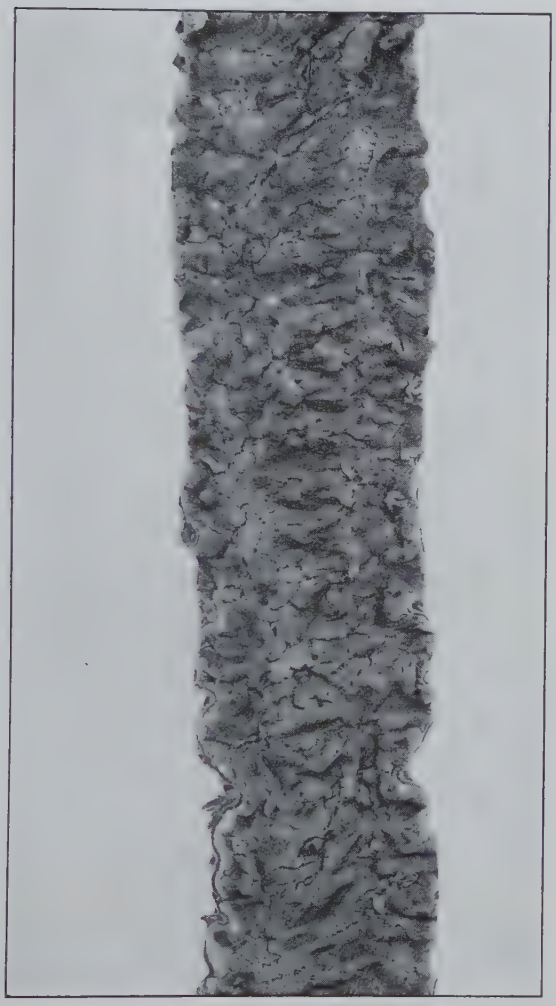


FIG. 4.—ACUTE AMOEBIC COLITIS.

Small undermined ulcers. (U. S. Army Medical Museum No. 39,113. Also from Haughwout and Callender.)

are undoubtedly primary pathogenic organisms, capable within themselves of invading the wall, although the host is commonly able to limit the extent of penetration. Whether they may be purely lumen dwellers is yet unsettled. Previous opinions on this question, even by those who may speak most authoritatively, are subject to error in the unknown. In considering the possibility it should not be forgotten that the amoeba may be grown apparently indefinitely as a "lumen dweller" in a culture tube. It should also be borne in mind that pathological findings in the intestine at autopsy in the hands of careful and experienced pathologists and in large services are not in proportion to the common relatively high incidence of the infection as reported from stool examination. Is it possible that even minute lesions which must be so commonly present unless this amoeba is frequently a pure lumen dweller should have been so completely overlooked?

The writer hesitates to acknowledge that in a considerable autopsy experience a condition in which there existed a special interest was not found. And yet such must be the case if *Endamoeba histolytica* always invades the intestine wall. Naturally the intestine has not been minutely examined always, nor have a great number of microscopic sections been done as a practice, but recently, at any rate, extra care has been exercised and if a common intestinal lesion, even small, has been overlooked it will take virtual serial section to reveal it. In this connection

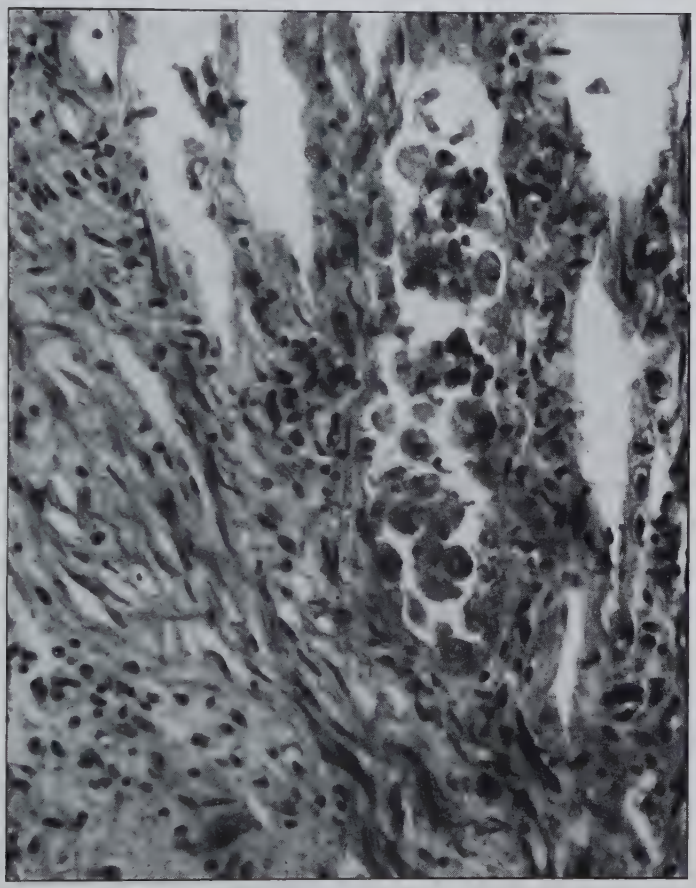


FIG. 5.—AMOEBIC COLITIS.

Nest of *Endamoeba histolytica* in the bottom of a gland crypt of the mucosa. Photomicrograph x 180.

it must be recorded that the writer's autopsy service is largely of negroes and that the incidence of the infection among them in this locality is unknown.

The contention of anything in the region of a constant intestinal invasion in the very large number of infected persons, if this itself is correct, remains to be proven.

The finding of an amoeba indistinguishable from *Endamoeba histolytica* as, apparently, a lumen dweller in the rat, must also be considered in an argument at the present.

The amoeba does not encyst within the tissues but this process occurs in the lumen as the organisms are carried down with the intestinal content to the lower bowel, where inspissation takes place. As they proceed to encystment they become smaller and less active. As cysts they pass out with defecation to become the form transmissible to a new host.

It is not known whether they multiply in the colon from their own cysts, that is, whether excystment takes place here. Ordinarily at least it appears that division of the trophozoites is the manner of increase and that this occurs mainly in the lumen and in the glands.

The properties of the amoeba which enable it to invade the tissue are its physical ability to squeeze between adjoining cells and, particularly, its cytolytic action upon cells with which it comes in contact (Craig, 1928). James (1927) (Scientific exhibits, Amer. Med. Ass'n) has shown that *Endamoeba histolytica* is capable of pushing its way between the epithelial cells of an intact mucosa. How common or important this is it is impossible to say. In the

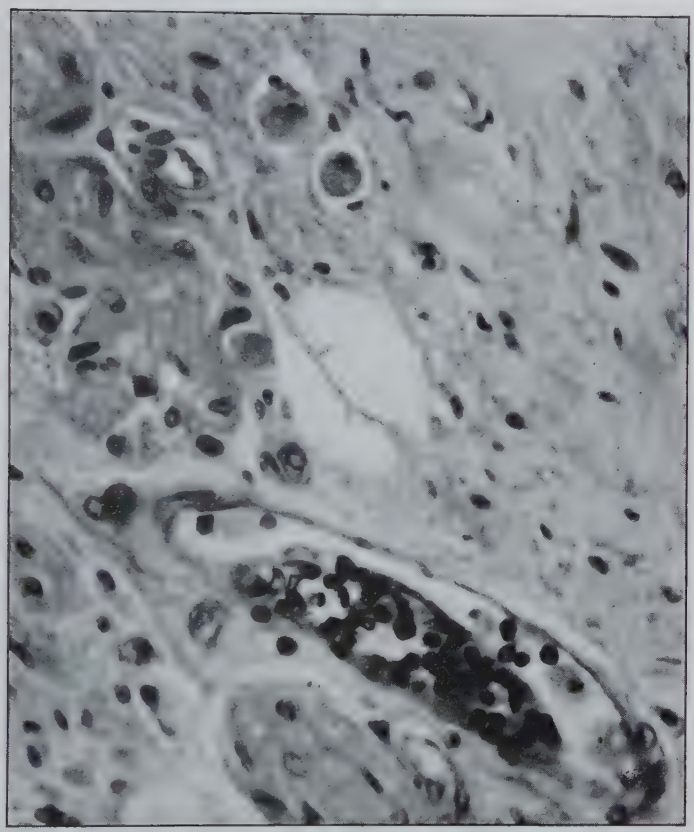


FIG. 6.—AMOEBIC COLITIS.

Endamoeba histolytica within a venule and in tissue spaces of the submucosa of the colon. Photomicrograph x 470.

writer's specimens from human amoebic colitis penetration of the surface epithelium has not been observed (Lynch, 1920). The amoebae do not occur in the superficial tissues of the mucosa. They invade the mucosal glands and single organisms may be seen

at different locations here. At the bottom of the crypts the amoebae appear to multiply, nests of them may be seen, and here the epithelium undergoes necrosis and dissolution, although in the upper part of the gland it may be intact and apparently healthy. Through the denuded base of the gland the amoebae penetrate and move to the deeper tissues, traveling through the lower tissues of the mucosa, the muscularis, the submucosa, and possibly through the entire wall. They spread laterally in the submucosa particularly, thus producing the characteristic lesion of amoebic dysentery with undermined mucosa, which may itself be more or less intact on the surface.

The resistance to their advance offered by the host is inflammatory locally. That there may be a humoral antagonism is indicated by Craig's (1928) complement fixation and Wagener's (1924) precipitin experiments.

The local inflammation includes congestion, exudation of plasma, infiltration of mononuclear leucocytes and proliferation of fibrous tissue. After exposure of ulcerated tissue to the intestinal bacteria the picture becomes confused by reaction against such infection.

The cytolytic activity of the amoebae is not conspicuous as a rule. As the parasites advance through the tissues, they may be seen rather far afield, where there is little evidence of tissue destruction or reaction. The necrosis and ulceration occurs behind them. Thrombosis of small blood vessels, which

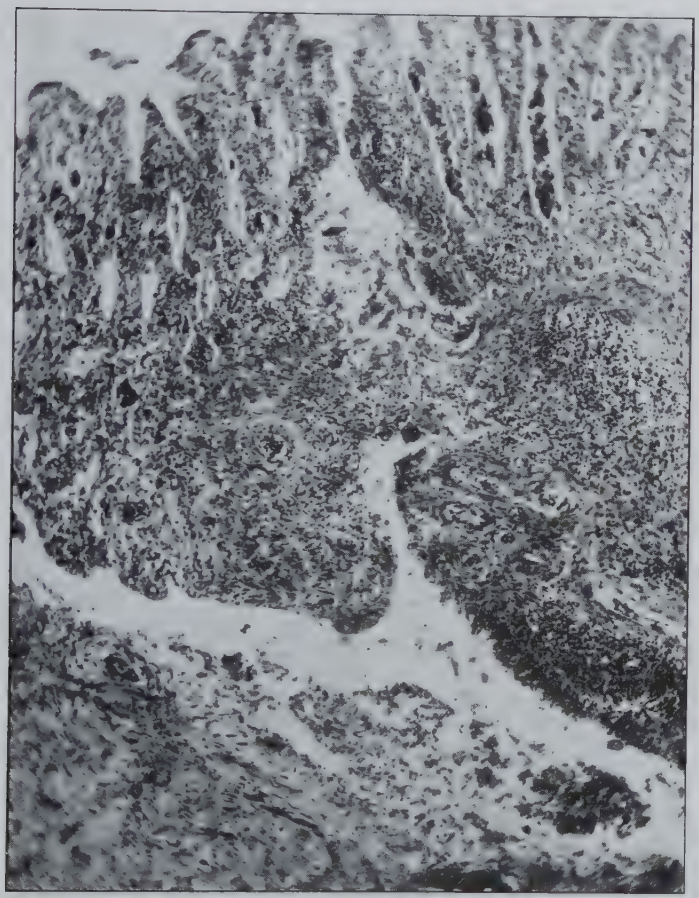


FIG. 7.—AMOEBIC COLITIS.

Undermining submucosal ulcer with sinus to the surface. Photomicrograph.

occurs as they are invaded, may be an important factor in the ulcer development.

Although the organisms invade the lymph spaces

and vessels, there is little tendency to infect the lymph follicles of the intestine or the lymph nodes of the mesentery. James (1928) found them in a

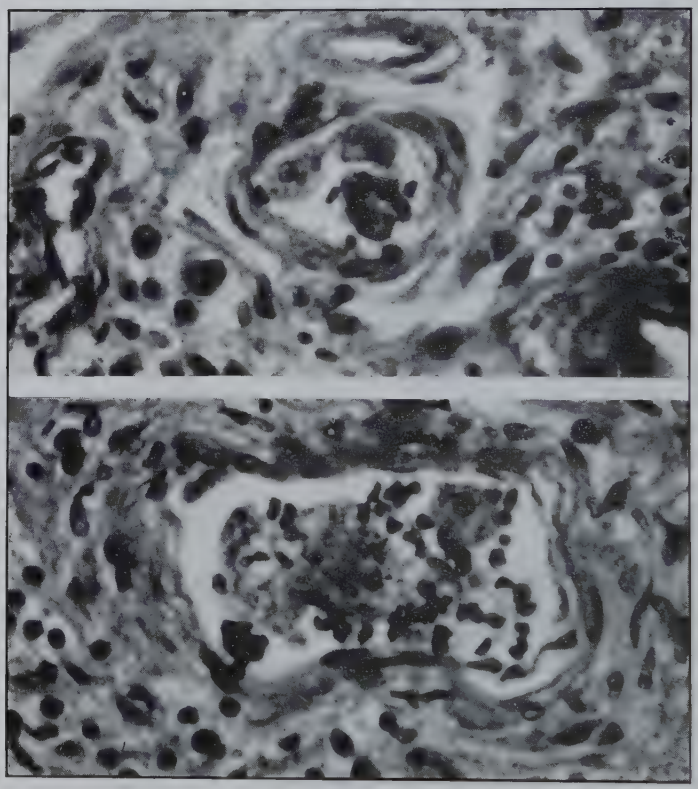


FIG. 8.—AMOEBIC COLITIS.

Endamoeba histolytica within small blood vessels of the submucosa, two in upper, one in lower, with thrombosis. Photomicrograph x 475.

lymph follicle of the human intestine, and Thompson (1926) has shown some invasion of lymphoid tis-

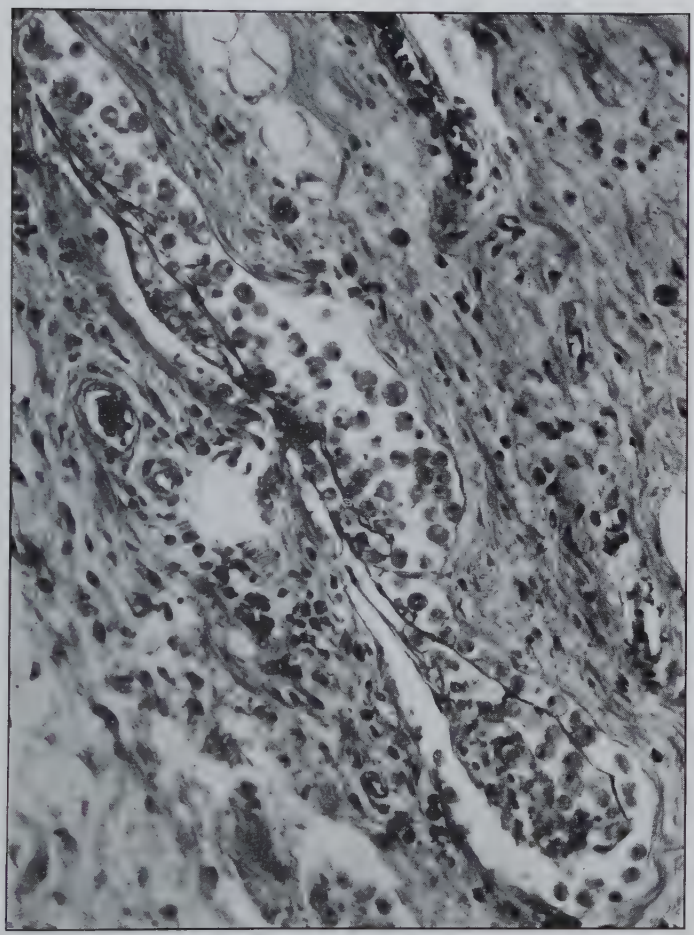


FIG. 9.—AMOEBIC COLITIS.

Large group of *Endamoeba histolytica* in a lymphatic vessel of the submucosa of the colon, with a few in the surrounding tissues. Photomicrograph x 195.

sue of the intestinal wall in experimental infection of the rabbit.

Their invasion of the blood vessels of the involved intestine explains the most common extension which occurs, that to the liver. Here some think they produce a not uncommon hepatitis. The well-known amoebic lesion in this organ is the abscess, single or

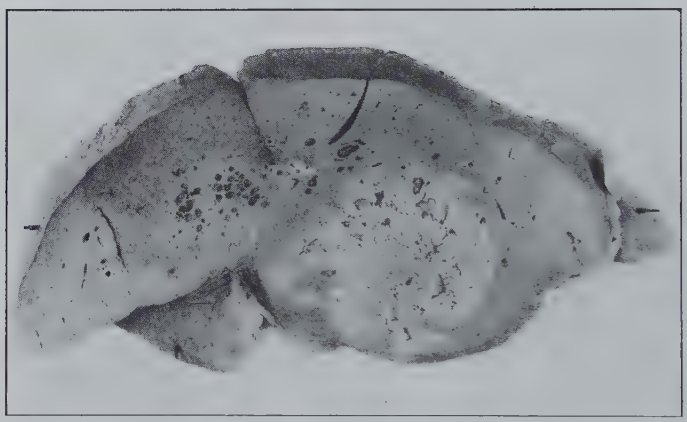


Fig. 10.—Amoebic Abscess of Liver.

(U. S. Army Medical Museum Specimen.)

multiple. The characteristic pathology of amoebiasis is in evidence in the abscess of the liver. The center of the focus of implantation undergoes necrosis and destruction, there is a mild type of reaction, involving hyperemia, plasma exudation, fibrin deposit, some mononuclear infiltration, with a few polymorphonuclear leucocytes, there being no gross or microscopic pus as seen is abscesses of bacterial production,

unless there occurs a secondary infection, no active abscess wall formation with conspicuous congestion, leucocytosis or fibrous tissue proliferation. The more chronic, the longer standing, the abscess, the more fibrosis occurs about it.

The amoebae spread peripherally between the cords of liver cells and as they advance the zone of liver cell degeneration and necrosis progresses until a large abscess reaching the surface may be produced. The extension of the process may lead to rupture into the abdominal cavity or even through the diaphragm into the chest and lung. Councilman and Lafleur (1891) in a masterly study of the pathology of amoebic dysentery, which placed intestinal amoebiasis on a definite pathological basis, describe a diffuse degeneration and necrosis of liver cells, especially around the central lobular veins, unrelated to the presence of the amoeba. This they believe to be the consequence of absorption of products of the amoebae in the intestine ulcers. This may be the hepatitis spoken of by clinicians during amoebic dysentery without liver abscess.

Abscess of the lung by direct extension is the next most common foreign extension, although blood born organisms may be transferred to these organs and to other parts, notably the brain. Wherever the amoebae go the characteristic lesion seems to occur; as the organisms spread the tissues become necrotic behind them, liquifying and producing the abscess

without leucocytes. The reaction to them is mild congestion, plasma exudation, fibrin deposit, mono-

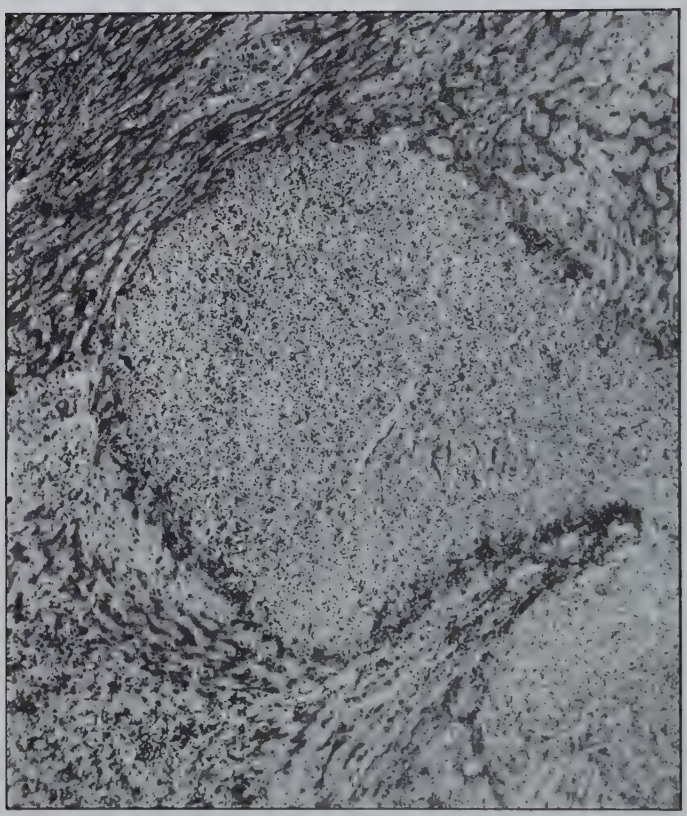


FIG. 11.—AMOEBIC ABSCESS OF LIVER.

(U. S. Army Medical Museum No. 44,739. Photomicrograph.)

nuclear cell infiltration, connective tissue proliferation.

Warthin (1922) has reported this amoeba in the

testis and its tract and Warsley and Bateman (1924), Craig (1911), Macfie (1916) and others have observed it in the urine. Kofoid (1925) and others have reported finding *Endamoeba histolytica* in duodenal drainage from cases of intestinal amoebiasis without liver abscess.

While it may escape the bounds of the intestine and the portal circulation, such accidents are unusual.

The finding by Kofoid and his co-workers of bodies which they took to be *Endamoeba histolytica* in the joints of arthritis deformans and the lymph nodes of Hodgkin's disease has not been generally accepted as correct. The writer has had no opportunity to attempt confirmation of their observations except in fresh lymph nodes of Hodgkin's disease. There no amoeba was found. Kofoid identified the cells which he found in these tissues by the number of chromosomes in the nucleus and by the mitosis of the dividing cell. One hesitates to question the correctness of observation by a protozoologist of Kofoid's calibre but there are so many pitfalls in such work that both biologists and pathologists will await further evidence and confirmation before passing judgment. The nature of the lesions in both cases, especially the character of the reaction in Hodgkin's disease, and the dissimilarity to the characteristic amoebic lesion, makes pathologists immediately skeptical that these diseases could be of amoebic origin.

It seems clear that the disease produced by this organism is virtually limited to the large intestine,

with direct extension to adjacent tissues, occasional excursion to the liver, and rarely accidental metastasis to other tissues.

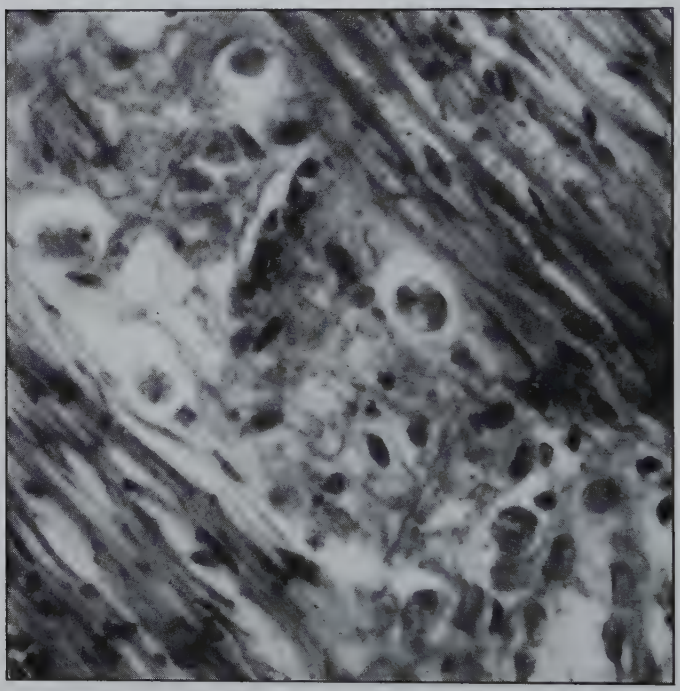


FIG. 12.—AMOEBIC COLITIS.

Group of *Endamoeba histolytica* within the tissues between the muscle coats of the colon. Photomicrograph x 475.

Local extension deep into the wall of the intestines is common in generalized colonic infection, usually with dysentery. Sometimes involvement of the peritoneum allows bacterial invasion to this tissue with local or general peritonitis but peritonitis is

more commonly the result of rupture of the ulcer through the intestine wall with escape of the content of the bowel.

Direct extension of the ulcers into the retroperi-

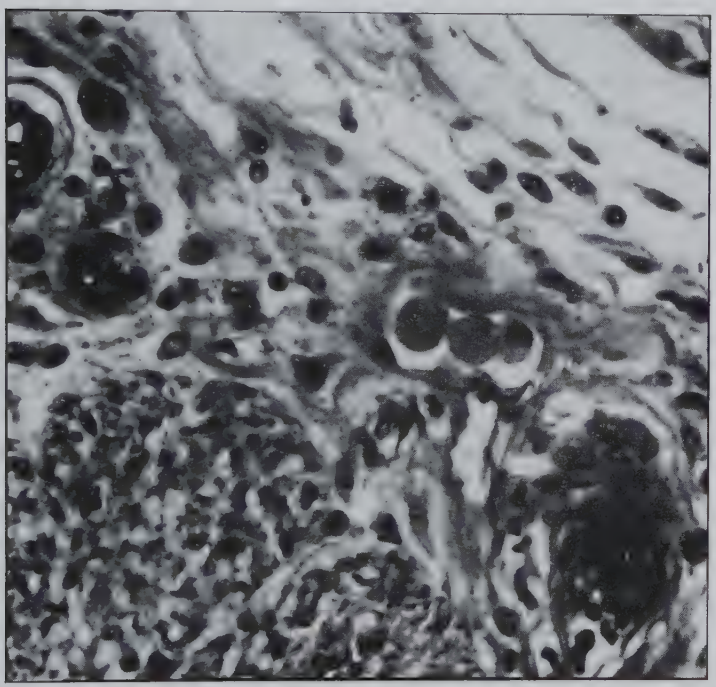


FIG. 13.—AMOEBIC COLITIS.

Three amoebae within a tissue space in the subperitoneal coat of the colon. Photomicrograph x 475.

toneal tissues, instead of through the peritoneal coverings, is not uncommon, with the progression of tunnelling suppurative sinuses extensively through the retroabdominal tissues, involving adjacent or-

gans. With this there is developed bacterial infection, a septic state, and death. This sort of extension has been more common in the writer's experience than others, even to the liver.

The disease of the intestine may be divided into an acute and a chronic stage, the latter by far the more common, the former the typical condition of amoebic dysentery.

It is convenient and permissible, both from the pathologic state and the clinical aspects, to divide intestinal amoebiasis into two phases, chronic intestinal amoebiasis and amoebic dysentery or acute amoebic colitis.

CHRONIC INTESTINAL AMOEBIASIS

This is the most common phase of the disease and covers, in our present knowledge, the carrier states. During its course or as its termination may occur a flare into the acute state of amoebic dysentery. During its course may also occur liver abscess, or rare accidental metastases elsewhere.

The clinical state is confused and not subject to a positive diagnosis without finding the amoeba.

Those of wide experience with the disease, notably James (1928) and Craig (1928), are convinced that it is of very important proportions in the class of those who suffer from long continued ill health with confusing symptoms of many kinds but particularly relating to the intestinal tract, "indigestion" as the term goes.

Although attacks of diarrhoea or even dysentery, alternating with periods of constipation, may be discovered in the personal history, they may not be more conspicuous than in other people of similar ailments. Constipation appears to be the rule of the intestinal dysfunctions. Vague abdominal pains, heaviness, minor tenderness over the colon, especially the caecum, flatulence, gaseous eructation, are the common abdominal complaints. Generally there is a condition of under weight, a "tired feeling", a lack of energy. There are headaches and possibly joint aches. The state of "neurasthenia" is the type, with subnormal temperature, low tension, cardiac irregularity, etc.

All of which furnishes a picture which is very common to the practicing physician.

The state is thought to be one of "toxemia" and Craig's complement fixation is an evidence of humoral absorption and antibody production.

Diagnosis is to be made by finding the amoeba in the stool, possibly only by repeated examination.

The pathologic state in this condition is supposed to be that of the small mucosal ulcer previously described. It appears that these ulcers may be only of microscopic proportions or, at least, barely visible on careful search. Presumably they develop slowly, heal, and develop elsewhere. These flask shaped ulcers may have the neck occluded by débris or mucus and may appear as yellowish nodules in the mucosa.

James thinks there is also a shallow surface ulcera-

tion, with only minor tissue penetration by the amoeba, but produced by their cytolytic substance. Here there is superficial necrosis and sloughing, the shallowness of the ulcer obscuring it from the casual examiner.

Interestingly, Councilman and Lafleur (1891) describe a superficial mucosal necrosis and softening of epithelium, along with the typical undermined ulcer in amoebic dysentery, which they believed to be produced by the cytolytic activity of amoebae on the surface.

While such a state of the surface epithelium in the acute form, amoebic dysentery, is consistent with the state of the intestine, it is difficult to relate it with a chronic condition. Further investigation will be required to show that such surface lesions are of amoebic origin and that they are a characteristic state of the chronic phase of the disease.

It is somewhat difficult at the present to evaluate the various findings and beliefs. It is, perhaps, significant that some of the most experienced workers who once believed that *Endamoeba histolytica* is an obligatory tissue invader, that its presence in the intestine always signified tissue destruction, have changed their minds and are now convinced that the organism may dwell only in the lumen at times.

All of which comes to the possibility that the symptomless "carrier" state of intestinal amoebiasis, possibly the condition of chronic intestinal amoebiasis, covers the large group of infections by *Endamoeba*

histolytica, where the amoeba is mainly or solely, as a common thing, a lumen dweller, occasionally becoming seated at the bottoms of gland crypts, where it may produce necrosis and enter the tissues, possibly destroying surface epithelium, as James observes, without penetrating below that. Certainly for the most part it must live and multiply in the lumen of the bowel if its numbers and prevalence are what they appear to be.

As a lumen dweller does it disturb the host? That remains to be answered.

The whole question of the state of the host in chronic intestinal amoebiasis and in the symptomless carrier of *Endamoeba histolytica* has yet to be settled; whether the vague disturbances of intestinal tract and of general health now attributed to this low grade infection are related to it. James believes that this state is exceedingly common, that it is of more importance to the human race than any other protozoal disease, even than malaria, that intestinal amoebiasis presents a disease state as protean in its manifestations as syphilis itself.

We have come, then, from the recent start of knowing amoebic dysentery as the disease produced by *Endamoeba histolytica,* to the point where this is recognized as the uncommon acute or relatively acute phase of the disease, while there exists in the population at large an incidence of latent infection conservatively estimated at from 5 to 10%, in many, if not all, of which it is the belief of careful and com-

petent experienced men that there is a chronic low-grade disease, of devitalizing quality but diagnosable only on finding the amoeba.

There is much yet to be learned about intestinal amoebiasis.

ACUTE AMOEBIC COLITIS (AMOEBIC DYSENTERY)

The acute phase of the disease has long been known as tropical dysentery. Although much more common in hot countries it is occasionally seen in other climates, in which it is more apt to occur in the summer. That the climate is directly related has not been determined. Certain tropical and subtropical countries are more prolific of intestinal amoebiasis because of hygienic conditions. Other conditions in hot countries are probably related to the production of these acute attacks, the devitalizing heat and humidity, possibly the food. Certain it is that intestinal amoebiasis in temperate or colder regions does not show this phase as commonly, even relatively.

There seems to be no proof that there are strains of the amoeba of different degrees of virulence, although some experimentation may have so indicated. In fact the work of Walker and Sellards (1913) is definite evidence against variations in virulence among different strains.

The resistance of the host to the infection appears to be of more concern, although as to just what is involved in that resistance we have little knowledge.

FIG. 14.—ACUTE AMOEBIC COLITIS.

Large undermined ulcers with sloughing. (U. S. Army Medical Museum No. 39,112. Also from Haughwout and Callender.)

The characteristic pathology of the condition is as described by Councilman and Lafleur (1891), with the undermining and tunnelling ulcer of the submucosa, distributed mainly in the caecum and ascending colon but not uncommonly involving the whole large intestine and extending over the ileocaecal valve. Penetration of the wall with perforation, peritonitis, extension into the meso-colon and retroperitoneal tissues, liver abscess, and rarely more distant amoebic abscesses may occur during its course.

As the condition becomes prolonged, healing of ulcers and the formation of fibrous adhesions between the intestinal coils and surrounding viscera may produce extensive matting of the abdominal viscera and deformity of the intestine.

The "moth-eaten" appearance of the colon at autopsy is typical and not to be confused with ulcerative colitis of other origin.

Among the symptoms of the disease, dysentery, with numerous blood-stained mucous stools, " bloody flux," is outstanding. It is reported that acute amoebic colitis may occur without dysentery and the characteristic stool, and I have done autopsies on cases dead of the condition, in whom there was no information of diarrhoea or dysentery. It is not improbable that dysentery has existed in some persons, the condition of whom at the time of observation precluded the possibility of obtaining correct information. Involvement of the rectum produces pain,

tenesmus, frequent small stools of glairy mucus with fresh blood, and straining at stool.

Disturbed digestion, tenderness over the intestine on pressure and colicky abdominal pains are prominent disturbances.

Systemic debilitation, loss of weight, weakness, vary with the severity of the attack. As it is prolonged they become prominent, and anemia, exhaustion and emaciation may be pronounced in the severe or fatal case.

Enlargement of the liver with or without sensitiveness varies in the experience of different observers. Early amoebic hepatitis may give an enlarged sensitive liver and a trace of jaundice.

Later amoebic liver abscess may produce a more pronounced state of a similar kind, probably with evening fever, chilly sensations or actual chills, and blood leucocytosis.

Proctoscopic or sigmoidoscopic examination may or may not reveal ulcers of the examined intestine. Negative findings here by no means exclude amoebic colitis, since there may be extensive involvement of the higher colon. Fever may or may not occur and when there is a blood leucocytosis it is probably due to a secondary bacterial infection.

An attack of amoebic dysentery may occur as the first evidence of intestinal amoebiasis or it may occur as an acute flare during the course of the chronic disease or as its end result.

In recovery from the acute attack there is gradual

convalescence, with decreasing symptoms, to the point of a few mucous stools a day, with tenesmus, disturbed digestion, anemia and emaciation, or merely the state of "neurasthenia" with indigestion, previously recited, in which constipation is prominent, or to a state of symptomless and seeming good health.

A concise conception of the pathology of the disease suffices to explain the clinical state fairly completely. It also is of great importance to a clear understanding of the reasonable expectations in the practical treatment of the phases of intestinal amoebiasis.

THE STOOLS OF DYSENTERY

While the stool of amoebic dysentery is characteristically of blood stained mucus there is a certain amount of possible error in depending on this gross appearance as a diagnostic feature. This has been emphasized by Haughwout (1924), by Haughwout and Callender (1925), and by Callender (1927), who have called attention to the practical value in diagnostic procedure of a microscopic study of the stool for other cells as well as for the organisms.

The writer (1917) called attention to the confusion which might occur between large phagocytic cells in acute non-amoebic dysentery (probably bacillary dysentery) and amoebae.

In bacillary dysentery there will be in the stool, which may be more or less blood stained and mucin-

ous, variable numbers of leucocytes, largely polynuclears, red blood corpuscles, and, particularly, large phagocytic endothelioid cells. These macrophages are practically non-motile as seen in the stool and contain ingested cells or cell particles. Whole leucocytes and red blood corpuscles or remnants of these ingested cells may appear in these phagocytes.

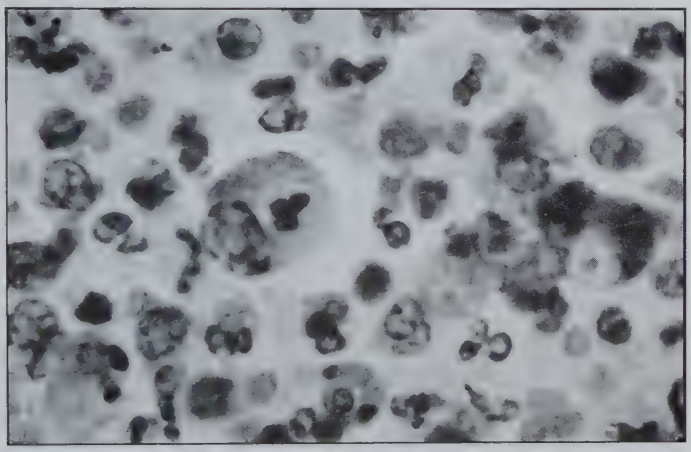


FIG. 15.—STOOL OF BACILLARY DYSENTERY.

Mononuclear phagocytes and leucocytes. (U. S. Army Medical Museum No. 41,158. Also from Haughwout and Callender.)

All phases of degeneration and disintegration of these cells as well as of leucocytes may be seen. The cellular content of the stool of bacillary dysentery is conspicuous.

On the contrary the stool of uncomplicated amoebic dysentery has few cells, possibly a few mononuclears and an occasional polynuclear. The body of tissue

origin which is conspicuous is the red blood corpuscle.

Uncomplicated amoebic dysentery does not pro-

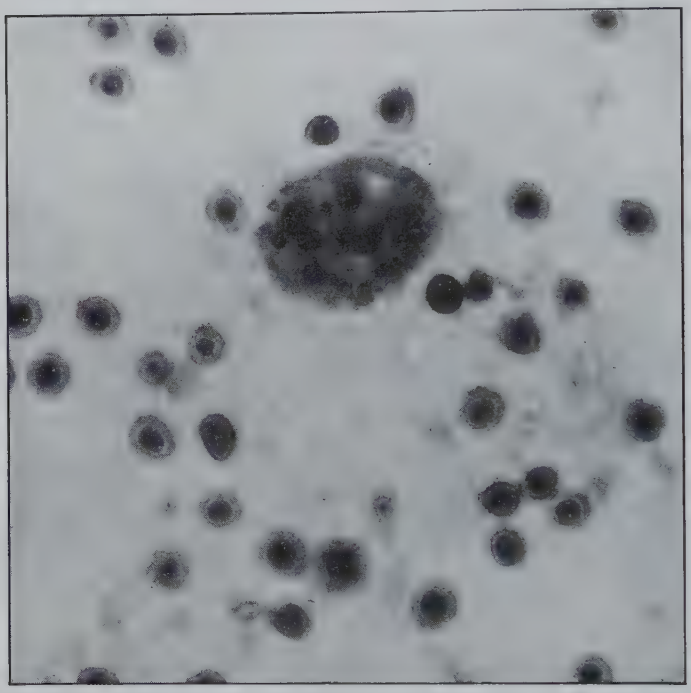


FIG. 16.—STOOL OF AMOEBIC DYSENTERY.

Red blood corpuscles and an amoeba with partially digested corpuscles. Photomicrograph x 1000.

duce the macrophage in the stool, while it is rather characteristic of bacillary dysentery.

In cases of the latter naturally there may be an amoebic infection. If there is an exudate as of bacillary dysentery in a stool containing *Endamoeba histo-*

lytica the indications of the bacillary dysentery are, of course, of the more immediate importance. The amoebic infection may be attended to when the bacterial infection is overcome or shown to not exist.

It is in such a stool that the active form of *Endamoeba coli* is most apt to be mistakenly identified as *Endamoeba histolytica* and the whole case misjudged.

When pus and these large phagocytic cells are features of the stool examination, utmost care should be exercised to avoid overlooking the occurrence of bacillary dysentery, particularly when an amoeba is present to confuse, and more especially when it is thought to be *Endamoeba histolytica.*

These are recommended as important practical considerations.

TREATMENT

In the opinions of practitioners of wide experience with the disease those who expect cure of intestinal amoebiasis or eradication of the amoeba from the intestine by any brief course of medication will come to disillusionment if they follow the course of their bona fide cases carefully and truthfully.

The first requisite to proper handling of a case of the disease is a correct diagnosis.

Since the dysfunctions of amoebic dysentery and chronic intestinal amoebiasis not uncommonly disappear spontaneously or without specific treatment

one must be guarded in speaking of bringing about a cure by any measure merely on the evidence of a return of the patient to apparently normal health. One may assist in relieving the disturbance of the disease but to cure intestinal amoebiasis necessitates the eradication of the amoeba from the host. Without ascertaining conclusively that this is the case one should not speak of a cure. This can be determined only by examining for the amoeba over a fairly lengthy period, bearing in mind that its numbers naturally vary and that after any ordinary therapeutic attack, especially if there has been recovery from the clinical state of amoebic dysentery, it may be very sparse for some time.

Absence of the amoeba from the stool by repeated careful examination, such as has been previously detailed, for a month or longer may be taken as reasonable proof of the eradication of the parasite.

The drug of longest specific use in the treatment of intestinal amoebiasis is ipecac. While ipecac itself is still used by some it has been largely replaced by its alkaloid emetine. This drug, although lethal to *Endamoeba histolytica* only when as strong as 1 to 10,000, according to Kofoid and Wagener (1925), exerts a powerful specific effect on the amoeba in the tissues of its host, it being particularly valuable in the active phases of the disease, usually controlling amoebic dysentery promptly. It is less effective against the "carrier" or the host who does not exhibit signs of tissue invasion. In that case a more power-

ful amoebicide is necessary, referring probably to the difference in location of the amoebae. The action of emetine appears to be rather indirect than direct on the amoeba.

The manner of administering the drug varies and this undoubtedly effects the success attending it. Emetine may be given hypodermically or orally or by both avenues simultaneously.

Dobell's (1921) method of giving emetine bismuthous iodide (double iodide of emetine and bismuth) is as follows:

Emetine bismuthous iodide is an insoluble brick red powder. It should be obtained from a trustworthy source and be guaranteed to contain not less than 26% of the alkaloid. The dose should be three grains daily, by mouth, for twelve consecutive days, not less. This may be attended by some nausea, but this can usually be controlled by a small dose of tincture of opium previously and by giving the drug after going to bed at night and not on an empty stomach. Dobell believes that this course will usually remove an infection with *Endamoeba histolytica* permanently, without giving symptoms of emetine poisoning. When it does not rid of the amoeba a further course should be given, three grains daily for twenty-four consecutive days or an ordinary twelve day course supplemented by emetine hydrochloride (½ to 1 gr. daily) hypodermically at the same time.

Wenyon and O'Connor (1917) advocate the combined oral and hypodermic administrations of eme-

tine hydrochloride. One grain of the alkaloid is given hypodermically in the morning and one-half grain in a keratin coated tablet at night by mouth, both every day for twelve consecutive days. James and Deeks (1924) recommend the use of emetine hypodermically with concurrent administration of bismuth by mouth. A routine in active amoebic colitis is as follows:

Give a preliminary dose of castor oil. Put the patient to bed and limit the diet to liquids, preferably milk. If tenesmus and griping pains are severe give a hypodermic of morphine and atrophine as indicated. Emetine hydrochloride hypodermically in ½ grain doses twice daily or 1 grain doses daily for 9 to 10 days. Bismuth subnitrate, a heaping teaspoonful (180–200 grs.) is mechanically mixed in a glass of water and taken every 3 to 4 hours. When the stools begin to form the bismuth is decreased to three times daily and a generous diet is gradually instituted.

Rectal irrigations of quinine, silver salts, iodine and other agents are unnecessary, according to Deeks, as are surgical measures for irrigating purposes.

These courses of treatment for active cases of amoebiasis take into account the stubborn nature of the infection and, particularly that of James and Deeks, the pathologic state of the intestine. It is not to be expected that the intestinal ulcers are to be healed in the periods of time covered by these therapeutic courses. One has only to picture the condition

to appreciate that. Consequently general measures aimed at allowing the best conditions for recovery of the intestine should be well borne in mind. For this purpose, apparently, is the use of bismuth. Such a diseased person should be governed by much the same rules as in any comparable ulcerative state of the bowel. Confinement to bed during the active phase of the disease and consistent with the physical state during convalescence is of importance.

The diet is probably of more consequence than one may now conclusively state, in fact there are indications that the diet may be an important factor in the aggressiveness of the amoeba. A bland liquid diet during an acute attack (Deeks specifies milk) and well balanced, nutritious, but not heavy feeding of non-irritating foods during the treatment of any form of the condition should be attended to. A reduction of the carbohydrates of the diet, sufficient protein, particular attention to green vegetables and fresh fruits, are to be recommended.

At the risk of being burdensome the writer would re-emphasize to practitioners that treatment is of both specific and non-specific kinds. The specific drugs must be given in sufficient dosage or long enough to kill out the amoeba. The non-specific is such as one would use for any similar pathologic state, remembering the nature, extent and location and the time required for the healing of such lesions.

Of the newer measures of treatment, that out-

lined by Cort (1928) emphasizes the present day idea that emetine is not to be depended upon to eliminate the amoebic infection but is most valuable in controlling the active phase of the disease. He recommends emetine in 1 grain dosage intra-muscularly daily for 7 days. After an interval of 1 week another series of 5 injections are given; then 2 series of 2 to 3 injections at intervals of 1 month. With frequent stools or loss of sphincter control, use chiniofon (yatren) enemas, one a day, with bismuth or opium, as indicated.

When the stools are less frequent give acetarsone (stovarsol) by mouth, (as well as emetine) one 4 grain tablet twice a day for not more than 6 or 7 days. Then continue with chiniofon (yatren) pills, first two 4 grain tablets twice daily, increasing to four pills three times a day. Repeat this course at the end of one month.

Cort considers emetine a specific for amoebic hepatitis but not for intestinal amoebiasis, which interpreted appears to mean that the effect of the amoebic products, from the ulcers of the intestine, upon the liver is counteracted in some way by the drug, that again the influence of emetine is more on the host than the parasite.

In Cort's experience abscesses of the liver should be aspirated and then treated with emetine, while in case of poor surgical risk on account of the amoebiasis, emetine may convert into a state favorable to operation.

In active phases of the disease when the patient is in bed or may be so confined, chiniofon (yatren) may be an effective drug, given in cachets of 3 grams per day for 10 consecutive days, accompanied by a liquid diet. It may also be used by rectum. It may produce diarrhoea itself.

In the experience of Jones and Turner (1929) yatren is a valuable drug in intestinal amoebiasis, particularly in dysentery, given in pills of 0.25 gms. each, coated, with phenyl salicylate, 1 gram by mouth three times daily during the first and third week, none in the second. No restriction of diet or confinement to bed is necessary. Children may be given a proportionate dose. There were no toxic manifestations but the drug may cause diarrhoea. The effect is claimed to be directly upon the amoeba in the intestine, and ulcers of the rectum healed in from seven to fourteen days.

Deeks (1928) reaffirms the excellence of his treatment with bismuth and emetine in amoebic dysentery, and says that the newer measures of treatment by bismuth-emetine-iodide, yatren, and stovarsol have not given equally good results.

Another valuable drug of comparatively recent introduction in the therapeutic armamentarium against amoebiasis is acetarsone (stovarsol), an arsenical preparation. Several arsenical drugs appear to be amoebicidal in much higher dilution than other so-called specifics for amoebiasis. According to Kofoid and Wagener (1925) the lethal dilution of stovar-

sol is 1 to 95,142, of neo-arsphenamine 1 to 142,000, of arsphenamine 1 to 100,000.

Stovarsol has the important advantage of being effective by mouth administration and without the necessity of putting the subject of treatment to bed. While it is of value in the acute case, it is of particular application to the ambulatory chronic case and the healthy carrier, according to Craig (1928). Symptoms of arsenic intoxication were obtained by giving one tablet (0.25 gm.) three times daily for a week, these consisting of gaseous abdominal distention, colickly pains, mild diarrhoea, and erythema. One-half tablet given three times a day for one week, with a cessation of treatment for one week, and a repetition of the course for the third week gave no untoward effects, and eliminated the amoeba in the majority of cases treated. Those not completely relieved by this course were so benefited by a second similar treatment. The subjects may be allowed to continue their occupations with dietary restrictions of rich foods and excessive carbohydrates.

In the consideration of all of which, the presentation of the several so-called "specifics" and the different measures of administration, lies the acknowledgment that the treatment of intestinal amoebiasis has yet to be proven as satisfactory as some have claimed.

Sometimes one measure and then another gives brilliant results, sometimes apparently none.

It must not be forgotten that the drugs are not

harmless themselves and that the course of treatment must be watched.

Finally, for re-emphasizing, the nature of the organism, its two phases, its habitat, in the lumen, in ulcers, in tissues, the local destruction of tissue and the necessity for producing healing, the possible extra-intestinal involvement, all must be considered in any rational medical attack upon the obviously diseased host or the apparently healthy carrier. Time and care are no small parts of the treatment.

CHAPTER V

The Amoebae (Continued)

Endamoeba coli. Morphology. Prevalence and Importance. Habitat and Effects. *Councilmania lafleuri.*

ENDAMOEBA COLI

The most common amoeba of the intestine of man, *Endamoeba coli,* was, according to Dobell and O'Connor (1921), first studied by Lewis (1870) and Cunningham (1871) in India. It is in prevalence, numbers, and size, the most prominent of this class of parasites.

MORPHOLOGY

The active form may vary from ten to forty microns in diameter but is usually between twenty and thirty. In the actively motile condition, taken fresh and warm from the contents of the higher large intestine, it is usually much less energetic than *Endamoeba histolytica,* moving about more slowly, not in such a "streaming" manner, and its pseudopodia are less prominent, less clear and not so "explosive." The ectoplasm and endoplasm are not so well divided and there may be but little difference except in the pseudo-

podia. However, it is not uncommonly seen, in favorable specimens of stool or in culture material, when these differences from *Endamoeba histolytica* are inconspicuous and when it defies positive identification by experienced observers. It is this fact which fre-

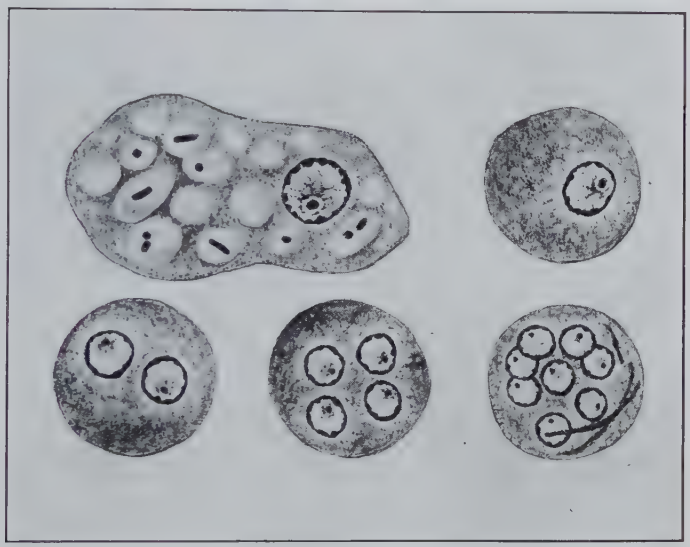


FIG. 17.—*Endamoeba coli.*

Drawings of trophozoite and cysts with one, two, four and eight nuclei, from specimens stained by iron-haematoxylon. x 1000.

quently produces an erroneous diagnosis of intestinal amoebiasis in the practice of medicine.

The nucleus of this amoeba, as distinguished from *Endamoeba histolytica,* is usually visible in the fresh unstained living and moving organism. It is seen as a round ring, not a vacuole, in the more granular and vacuolated endoplasm. It flows about during

the progressive motility of the amoeba in a passive way, along with the other inner contents. It is best seen as the amoeba changes its position, when there is protruded a long pseudopod into which flows the endoplasm. As the nucleus moves into the forward pseudopod it may be plainly seen.

The endoplasm in this phase is usually grossly vacuolated and contains numerous food bodies of a variety, bacteria, yeasts, *Blastocystis,* crystals, and even the cysts of other protozoa. These objects give evidence of its natural food supplies. In contrast, *Endamoeba histolytica* seldom contains bacteria and probably never consumes these other objects commonly seen in *E. coli.* As a rule *Endamoeba coli* does not eat red blood corpuscles; in fact it seldom has the opportunity, but it may apparently do so under certain conditions. The writer (1924) incubated at 37.5°C. portions of fresh stool containing large numbers of sluggish amoebae in test tubes containing 0.9% sodium chloride solution to which was added a small amount of fresh human blood. Within two hours the amoebae became very active in motility and had ingested many red blood corpuscles. Subsequent repeated examination of the stools of this person never revealed any but an eight nucleated amoeba cyst, identified as *E. coli.* The only question arising about the identification of the amoeba of this experiment is raised by Kofoid and Swezy's (1921) description of a new genus, *Councilmania,* the species of the human intestine named *Councilmania lafleuri,*

which as yet has not been well accepted by protozoologists and which must be held *sub judice*. These investigators use the phenomenon of phagocyting red blood corpuscles as one of the main features of differentiating the active forms of *Endamoeba coli* and their *Councilmania lafleuri*. The particular features of difference between the two as specified by them are the "dispersed" karyosome of *Councilmania* as contrasted to the compact form of that of *E. coli*, and the reproduction by budding from the cyst of the former. The dispersed karyosome was present in some of the cysts of the amoeba encountered in this experiment but the process of budding was not seen at any time. Should Kofoid's contention be accepted this experiment will no doubt be considered to affect *Councilmania lafleuri* and not *Endamoeba coli*.

Dobell and O'Connor (1921) say of *Endamoeba coli*, that the food "vacuoles never contain red blood corpuscles," while Wenyon and O'Connor (1919) state, "If amoebae containing red blood corpuscles are present in the stool—they are *Endamoeba histolytica*." This long accepted feature of difference between *E. coli* and *E. histolytica* must be held subject to question as a positive means of differentiation for the present.

When the amoeba is obtained from the lower colon or in the ordinary stool it is proceeding to encystment or is already encysted. The "precystic" form is smaller, it has stopped feeding, it is sluggish or motionless. If it shows pseudopodia they are small, its

endoplasm is granular, and there is no sharp delineation of ectoplasm. In this small sluggish precystic form it is not safe to attempt differentiation from *E. histolytica* in unstained specimens, nor probably in stained preparations except in expert hands.

The cyst is the form to be positively identified by any who can find these bodies and can count the nuclei in unstained or stained preparations. The cysts usually show in characteristic appearance in formed stools, measuring from ten to thirty or more microns in diameter but usually between fifteen and twenty. They are larger than cysts of *E. histolytica* as a rule. They are rounded, have a rather thick wall, the body is finely granular or hyaline in the living cyst and they have typically eight nuclei. Chromatoid bodies are not conspicuous in the mature cyst. They appear as splinters or slivers or filamentous lines. Sixteen nuclei, or even thirty-two, may be seen. In the immature cysts glycogen is prominent, especially in the iodine preparation, the nuclei are larger and the chromatin more prominent, the chromatoid bodies are more common and more numerous, and the nuclei are one, two, or four in number. The writer (1924) has observed *Endamoeba coli* (unless it be *Councilmania*) which showed constantly a chromatoid body of round form about the size of the mature nucleus, in the cyst.

The nucleus in *E. coli* is characteristic and may usually be seen in the living organism at any stage. In the very active streamingly motile form it may be

invisible. It is seen unstained as a refractive ring of granules, and the number of nuclei may be commonly counted in the living cyst. This, however, is facilitated by the iodine preparation previously described. The particularly important and practically useful point about the cyst is that any amoeba in the stool developing a cyst with more than four nuclei is not *Endamoeba histolytica*. Let medical laboratorians adhere to this point as an anchor.

Stained by the iron-haematoxylon procedure the nucleus of both active and encysted forms is round, has a definite rim, to which rather large chromatin particles cling in irregular distribution. The karyosome is large and not in the center, although it may appear so when the cyst is viewed in a certain exposure. It is eccentrically situated. This body is compact, although, unless *Councilmania* is valid, it may appear as a group of small granules, and it is surrounded by a "halo." The nuclear network shows a few granules of chromatin to the expert examiner.

It may, therefore, be readily seen that to the experienced there are morphological differences between the nuclei of *Endamoeba coli* and *Endamoeba histolytica* upon which positive differentiation may be made. To the inexperienced observer these features are not so clear and unless they are they furnish no help in diagnosis.

In iron-haematoxylon stained specimens the trophozoite is seen beautifully prepared for observation of it parts and ingested food.

PREVALENCE AND IMPORTANCE

Endamoeba coli is by far the most common intestinal protozoon. A large per cent of the population of any place, if not the majority, will show it on thorough search. It is an indication of the commonness with which the food of man is contaminated with the excreta of his fellows. Some believe that virtually everyone harbors this amoeba at some time.

Whether it may spontaneously disappear from the intestine is unknown. The writer knows of one person who has shown it on numerous examinations over a period of fifteen years.

Its particular importance lies in the common confusion of it in medical practice with *Endamoeba histolytica.* The writer knows of a number of instances in which physicians have treated and "cured" cases of intestinal amoebiasis or amoebic dysentery, so diagnosed, in whom *Endamoeba coli* was the only amoeba present and in whom it remained after the "cure."

HABITAT AND EFFECT

Endamoeba coli inhabits the lumen of the first part of the colon in its actively feeding stage. As it passes down the colon it encysts and passes out in the feces, from which it is transferred to a new host by ways previously discussed in the case of *Endamoeba histolytica.* In the intestine of its new host it is liberated from the cyst and completes its division

into eight offspring, from which point the life habits and cycle are repeated.

It has never been known to invade the tissues or to do anything harmful to its host. Other animals may harbor amoebae of similar characteristics but, so far as is known, they are distinct.

EXPERIMENTAL INFORMATION

Growth of *Endamoeba coli* in culture has been reported by Kofoid and Allen (1926) and by St. John (1926).

COUNCILMANIA LAFLEURI

To Kofoid and Swezy (1921) are due the credit for either discovering a new genus of amoebae, parasitic in the intestine of man and other animals, or of introducing another element of confusion into an already complicated situation. In the status of this question at the present time it is largely Kofoid and his co-workers against the field of other protozoologists. The time is not yet when the controversy may be settled or when one who has not been a material worker on this particular question should bear any but an open mind. Neither is this the place to analyze the data in detail. The question is an important one in medical protozoology because of the suspicion that *Councilmania lafleuri* may be harmful to its host. It is considered sufficient to state the problem at this time and to refer those who may

be interested in the details to the reports of the California investigators and of those who deny the correctness of their observation.*

Councilmania lafleuri, the species of the intestine of man, is described as being of an activity in mo-

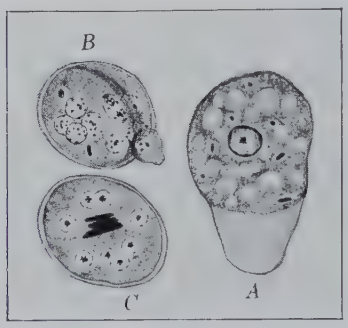


FIG. 18.—*Councilmania lafleuri.*

As shown by Kofoid and Swezy. *A,* trophozoite. *B,* budding cyst. *C,* cyst. (Univ. of California Press.)

tion comparable to *Endamoeba histolytica* and as readily ingesting red blood corpuscles. The wall of the cyst is very thick and the shape of this body is

* See: Kofoid, C. A., and Swezy, O., Univ. of Cal. Publ. Zool., 20, 169, 1921. Kofoid, C. A., Swezy, O., and Kessel, J. F., Ibid., 20, 431, 1923. Kofoid, C. A., Swezy, O., and Kessel, J. F., Ibid., 26, 21, 1924. Kofoid. C. A., and Swezy, O., Journ. Parasit. 10, 153, 1924. Kofoid. C. A., Proc. Soc. Exp. Biol. and Med., 23, 299, 1926. Allen, E. A., Univ. of Cal. Publ. Zool., 29, 175, 1926. Wenyon, C. M., On Three Supposed New Entamoebae of Man. Trop. Dis. Bull., 19, 19, 1922. Gunn, H., Councilmania lafleuri not a new amoeba. Journ. Parasit., 9., 24, 1922. Wight, T., Notes on Councilmania lafleuri, Proc. Soc. Exp. Biol. and Med., 22. 517, 1925. Wight, T. and Prince. L. H., Artifacts in Endamoebae which have led to the Naming of a New Genus and Species. Am. Journ. Trop. Med., 7, 287, 1927. Wenyon, C. M., *Protozoology.* Vol. I. New York, 1926.

not as definitely rounded but of the same general appearance as *E. coli.* The nucleus has a thin layer of peripheral chromatin and the karyosome is "dispersed" instead of compact. The particular characteristics upon which it seems that the validity of the genus must rest are: (1) the reproduction by budding from the cyst of eight offspring through a pore on a chromaphile ridge, and (2) the number of chromosomes, said to be eight as contrasted to six in *E. coli.*

Gunn (1922), Wenyon (1922), Wight and Prince (1926), and others have denied the existence of the process of budding, they having observed similar processes occurring from cysts of *E. coli* as artifacts, from pressure or other physical deformations, and at least Wenyon (1926) questions the possibility of consistent reliable counting of chromosomes in the dividing nucleus of amoebae.

Kofoid and his co-workers continue to adhere to the specific points which they have reported. They have also reported cultivation of *C. lafleuri,* in which it remained distinct from *E. coli,* encystment in culture (Allen, 1926), the occurrence of species of *Councilmania* in rodents (1923), the observation of cysts of *C. lafleuri* in duodenal drainage, the occurrence of two additional species in the human intestine (1928), and an incidence of 7.8% in 4763 persons on 20,264 examinations (1924).

The writer (1924), as previously recited, reported upon an intestinal amoeba which had the character-

istics of *Councilmania,* except that no budding was seen, nor were chromosomes counted, which ingested red blood corpuscles in culture tube.

Artifacts of the cysts of amoebae are certainly very common, although nothing resembling budding of amoebulae is often seen. The general characteristics of the species are common in amoebae identified as *Endamoeba coli* at the present. As far as the number of chromosomes is concerned, Wenyon's difficulty is no exception. The burden of proof still rests with Kofoid and his adherents but if the budding process was correctly observed it will undoubtedly be only a matter of time until the controversy is cleared. Further, if the number of chromosomes in the dividing nucleus may be accurately counted, and, of course, they will be, this will be a point of mathematical calculation not subject to personal equation. That time is not yet, however, certainly not for the average experienced observer. The number of chromosomes in the nucleus of amoebae, identified as such by Kofoid, in the lymph nodes of Hodgkin's disease and in the bones of arthritis deformans, has been used as the main point of identification of *Endamoeba histolytica* in these instances. It is a very important point of cytological distinction and further work will be of great interest.

CHAPTER VI

The Amoebae (Continued)

Endamoeba gingivalis. History and Relation to Pyorrhoea Alveolaris. Morphology. Habitat and Effects. Experimental Information. Prevention and Treatment.

ENDAMOEBA GINGIVALIS

The common amoeba of the human mouth is *Endamoeba gingivalis,* discovered by Gros (1849) as the first known parasitic amoeba of man. Since that time is has been rediscovered several times; by Steinberg (1862), by Grassi (1879), by Prowazek (1904), and by others. It has naturally been given several names by its discoverers, among which *Amoeba dentalis* and *Entamoeba buccalis* are still used, although erroneously, at times.

At least as early as 1913 Professor Allen J. Smith of the University of Pennsylvania was interested in the relation of this amoeba to pyorrhoea alveolaris and was working on the problem. Associated with him was Dr. M. T. Barrett, a dental practitioner. He later had associated with him in the study of *E. gingivalis* in the tonsils Dr. W. S. Middleton. In 1914 Barrett published a preliminary account of this

work, in which the positive occurrence of *E. gingivalis* in forty-two cases of pyorrhoea and the absence of it in normal mouths was reported. This report of Smith and Barrett states a belief in the pathogenicity of the amoeba on account of its constant association with the disease, its absence from normal gums, its tissue cell inclusions, and the elimination of the amoeba and improvement of the disease by treatment with emetine.

Chiavaro (1914) published a report immediately following this, in which he ascribed no etiological rôle to the amoeba which he observed in twenty-two cases of pyorrhoea and a few out of a number of cases of dental caries.

Also in 1914, Bass and Johns recorded the presence of *E. gingivalis* in pyorrhoea in eighty-two cases. These investigators (1915A and 1915B) later published a report of three hundred cases of pyorrhoea harboring the amoeba and also a book presenting their observations and ideas on the subject. They were convinced of the etiological relation of the amoeba to pyorrhoea alveolaris and of the relief to the infection and the progress of the disease attending treatment with emetine.

Further investigation by Smith, Middleton and Barrett (1914), by Smith and Barrett (1915) and by Evans and Middleton (1915) enlarged the field to include consideration of the presence of *E. gingivalis* in the tonsils, and the suggested relationship of systemic complications such as arthritis and anaemia.

Spectacularly hopeful as was the rise of oral amoebiasis, especially in relation to common pyorrhoea alveolaris, its downfall was equally but disappointingly rapid and spectacular. Following the reports of the Philadelphia and New Orleans investigators the dental and medical professions, in common parlance, took "hook, line and sinker." Emetine and alcresta ipecac were to eradicate pyorrhoea, hitherto and now either most difficult or unmanageable, and the speculated effects upon systemic states of disease were many. At the present time, the theory of oral amoebiasis and its cure by specific treatment is practically totally discounted.

The writer (1915), while not doubting that *E. gingivalis* was related etiologically to pyorrhoea, reported its presence where it was obliged to be a harmless dweller, and also in ulcers of the mouth and tongue where it was probably only a contaminant. It was also recorded that the ipecac treatments so enthusiastically recommended at the time had failed to eliminate the amoebae from some pus pockets and to bring about a cure of cases of pyorrhoea. A warning was sounded as to the constant pathogenic relationship of the amoeba, the necessity of considering other factors, and the unsoundness of expectancy of "cure" in certain cases of Rigg's disease.

Under the conditions of the occasion a bona fide specific cause and treatment would have suffered a reaction, perhaps similar to that which occurred. There was so much "chaff" in general dental and

medical practice that if there was any good at all it became hopelessly lost. Soon began to appear adverse reports as to the relation of the amoeba to pyorrhoea and the failure of the suggested treatment and it was not long before the whole idea was generally abandoned.

It would seem remarkable indeed that those who were instrumental in the rise of oral "endamoebiasis" should have been so completely in error in their observations. It is difficult for the writer to believe that some of what he saw in this work was unreal. It is suggested—with timidity—that the canons of oral amoebiasis have not been closed, that somewhere in this work was something real which will yet be uncovered from the avalanche of reactionary report.

THE AMOEBA

The amoeba of the mouth, which may be found in fresh preparations in the living and moving state, and whose structure may be studied in detail by the iron-haematoxylon staining method previously outlined, is most easily found in thin smears stained as an ordinary blood film, by Wright's or Leishman's stains. In the fresh warm motile state it reminds one of *Endamoeba histolytica*, having a similar progressive motility. Ordinarily it ranges from ten to twenty-five microns in diameter. In fresh preparations it may be seen to emerge from the particles of débris with which it is in intimate association and

to move across a clear field in rapid progression. Its ectoplasm is clear and hyaline, its pseudopodia hyaline, broad, and frequently thrust out in a sudden manner. The endoplasm is granular and food particles may usually be seen in it. The nucleus is usually not to be seen in such specimens.

Stained with the blood film stains the amoeba is

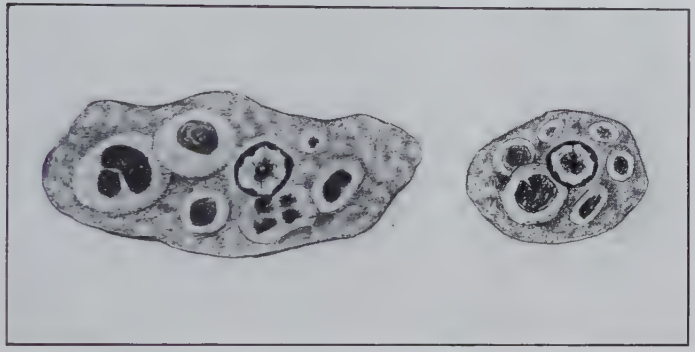


FIG. 19.—*Endamoeba gingivalis.*

Drawings of specimens stained by iron-haematoxylon. x 1200.

conspicuous. The ectoplasm and pseudopodia are deep blue, the endoplasm is lighter blue and contains purplish nuclear particles of ingested body cells and possibly pink erythrocytes or remnants. While the nuclear detail is not brought out in such preparations it may be seen as a red body. Variously stained bacteria occur in vacuoles in the endoplasm.

The nucleus is best studied in iron-haematoxylon preparations, which also reveal the parts and ingested objects of the parasite. The nucleus is rounded, there

are irregularly distributed chromatin granules on the rim and a central or near-central karyosome.

For the finer details of morphology the reader is referred to Kofoid and Swezy (1924).

The existence of cysts has not been conclusively shown. It is not unlikely that the conditions of its environment, in which there is no comparable regular change and inspissation of its medium, such as is associated with intestinal amoebae and related to their encystment, and, also, the literally direct ready transmission from one host to another, have made it unnecessary that this protozoon should have such a resistant protection. If it ever encysts it must be under rare and unknown conditions.

HABITAT AND EFFECT

Endamoeba gingivalis is practically a universal inhabitant of the mouth of man at some time during an average lifetime. All people who have any degree of pyorrhoea alveolaris harbor it, and most people past early adult life have some grade of this condition.

It may be found in the crypts of the tonsils, in ulcers of buccal mucosa or the tongue. Generally speaking it does not occur in the normal mouth but it may and commonly does occur whenever there is a recess or pocket in which it may grow and accumulate. The writer (1915) recorded finding it on the false teeth and between them and the gum of two persons who were completely toothless, with healed

gums and alveolar processes. In ulcers of buccal mucosa and tongue, where it was observed (Lynch, 1915), its appearance and disappearance seemed to bear no relation to the development or progress of the pathological state. It was evidently a secondary invader there.

The amoebae may be found in any grade of inflammation of the gums, from that grossly inconspicuous but showing a tendency of the gingiva to bleed unduly and microscopic pus behind the margins, to the chronic suppurative inflammation with pus pocket formation and receding of the gum margins, known as pyorrhoea alveolaris, in all of its stages of advancement. It occurs where there is exudation from inflamed tissue and a harbor for its lodgment.

In the depths of the pus pockets it flourishes, as was shown by the writer (1915), by Bass and Johns (1915), and more recently by Kofoid and Hinshaw (1929), who, according to Kofoid (1929), studied the amoeba in relation to its whole environment by section of the pus pocket and surrounding structures in toto. According to these investigators the amoebae occur about the filamentous border of leptothrix and other similar bacteria which have undergone calcification to form the tartar. Here they feed on the nuclei of the disintegrating pus cells. They are very numerous and form a heavy layer in the outer free filamentous covering of the solid serumal tartar, being most abundant about the point in the deeper part of the pocket where the tartar is thickest, and extending

some distance below the tartar adjacent to the cementum. They do not invade the gingival or bony tissues but the latter between the teeth and below the level of tartar formation is extensively absorbed. This report expresses the observation of the present writer in failing to find invasion of the tissues by the amoeba.

Kofoid (1929) develops a very pretty chain of argument from his studies, leading to the inference of a part in the events of pyorrhoea alveolaris played by *Endamoeba gingivalis*. In the first place is the infection by leptothrix and other filamentous bacteria, as a basis for the tartar formation by their calcification. Next comes the amoebic infection which is able to maintain itself on the fringes of the tartar calculus. There the amoebae consume leucocytic nuclei and excrete from these nucleo-proteins, phosphorous, available for deposit in the tartar as calcium phosphate. The bone of the adjacent teeth is resorbed near the site of the deposit of the calcium carbonate and phosphate by some unknown process. Thus one is led to suspect the triad, bacteria, amoebae, calculus, against which the more successful dental measures of instrumentation and surgery in the treatment of pyorrhoea may be directed, although unconsciously.

The amoeba not only ingests nuclear remnants of pus cells but it may consume red blood corpuscles (Smith and Barrett, 1915, and Howitt, 1925) and may even haemolyze these elements without ingesting

them. When erythrocytes are ingested they are rapidly destroyed, thus apparently accounting for the fact that they are rarely recognized in the amoebae.

Ohira and Noguchi (1917) probably first cultivated *Endamoeba gingivalis*, in a medium of ascitic fluid and Ringer's solution in equal parts. If so, this was the first successful cultivation of a parasitic amoeba. It has been extensively cultivated by Drbohlav (1925), by Howitt (1925), and by Dobell (1926), a favorable medium for its growth being the L. E. A. preparation of Boeck and Drbohlav (1924) as modified by Drbohlav, described in the cultivation of *Endamoeba histolytica*. Howitt recommends the use of 0.25 grams of glucose instead of 2.5 grams, as originally given, in making up Locke's solution.

In culture Howitt (1926) found that emetine in dilution of 1 to 25,000 diminished but did not eliminate the amoebae and a few were alive in dilution of 1 to 1660. Stovarsol was the most effective amoebicidal agent used.

Koch (1926) found acriflavine in dilution of 1 to 200,000, acridine yellow R 1 to 90,000, and aurimine, diluted 1 to 60,000, lethal to the amoebae. These dyes were not injurious to the gums of rabbits and it was suggested that they might be used in the human mouth. Koch (1927) also found the amoeba to be resistant to lower temperatures than that of the body, while it could be killed by a temperature of 55°C. in two minutes, by 52°C. in ten minutes, and

by 45°C. in twenty minutes. The organism could withstand apparent drying for about three minutes, showing the possibility of droplet transmission.

Kofoid (1929) reports that in his laboratory dogs have been successfully infected experimentally with *Endamoeba gingivalis.* Of these animals only those with gingival inflammation, pocket formation or loose gingivae at the time of inoculation, were susceptible. He found all of the monkeys which he examined to harbor an amoeba indistinguishable from *E. gingivalis,* those having the heaviest infections showing a condition closely resembling pyorrhoea in man. It should be noted that Goodrich and Moseley (1916) found an organism indistinguishable from *E. gingivalis* in the mouths of dogs having a similar condition to pyorrhoea in man. The writer has been unsuccessful in attempts to plant the amoeba from the human mouth directly to the gums of guinea pigs, rabbits, and dogs, all animals used having apparently normal gums and teeth.

PREVENTION AND TREATMENT

At the present time the treatment of oral amoebiasis cannot be very intelligently discussed. Man is universally subject to the infection very commonly. It is undoubtedly transmitted from person to person by direct or almost direct mouth passage, probably in large part by the act of kissing. Were it desirable to prevent this infection avoidance of such direct contact might be very effective. Indirect passage by means of the cup, spoon, fork, or other like

used object, contaminated by an infected person and on which the amoebae had not been killed by complete drying, may be a factor in its spread. The immersion of such objects in water at 55–60°C. or above for a few minutes would render them amoeba free.

Of mouth washes, that of emetine would seem necessarily ineffective. Those desiring such a solution of apparently amoebicidal properties should try stovarsol or such of the dyes used by Koch as may be conveniently used in such a manner. Anyone who expects to eliminate an established infection by means of mouth washes will be disappointed.

As to any further specific treatment of pyorrhoea, the writer hesitates to make such a recommendation except as an experimental measure. In the heyday of treatment with emetine, suppurative disease of the gums with small pocket formation was seen to improve to the point where there was no pus to be found, without the use of any dental instrumentation or other adjuvant except careful cleaning of the gums and pockets. In the early stages of such a condition one half grain of emetin hydrochloride was given daily by hypodermic injection until after the pus and amoebae had disappeared. In what was called the early stages of chronic pyorrhoea, with chronic catarrah of the gum margins, with contraction, soreness and tendency to bleed, and the formation of pus pockets beneath, it was recommended that the emetine treatment be combined with careful regular dental instrumentation until not only the

amoebae and pus had disappeared but the tissues had healed and become healthy. Otherwise recovery could not be expected and recurrence of amoebae took place.

In late pyorrhoea, particularly with deep pus pockets and extension sinuses, extensive tissue destruction, particularly of the supporting and adhering tooth attachments, it was recognized that it was most difficult or impossible to eliminate the amoebae and that lost tissues could not be fully restored. The extraction of loose teeth, surgical opening and drainage of deep sinuses or pockets, the syringing of pockets with water or saline solution followed by flushing with 0.5% emetine solution, thorough and regular dental cleaning, in addition to emetine hypodermatically, were recommended as measures directed at halting the progress of the disease, but not expected to be curative measures.

As a research procedure it is needed now to revive an interest in the treatment of oral amoebiasis in the light of some of the information which has been gained since the abandonment of the whole idea. Stovarsol both locally and taken by mouth as in intestinal amoebiasis should be tried. Chiniofon and the dyes of Koch and the application of heat, these and other measures, associated with dentistry and dental surgery, are available for experimentation. But let all be most carefully controlled and calculated. Let us not have another spectacle such as occurred about 1915–1916.

CHAPTER VII

The Amoebae (Continued)

Endolimax nana. Iodamoeba bütschlii. Dientamoeba fragilis. Other Amoebae

ENDOLIMAX (ENDAMOEBA) NANA

This small amoeba was long confused with other parasitic and free living forms. Although it is a very common amoeba of man and had been observed by several protozoologists before that time, it was first described by Wenyon and O'Connor in 1917. Its main interest lies in the fact of its commonness and the liability of confusion with *Endamoeba histolytica.*

As it occurs in the stool it is inconspicuous in any but very careful study. It is usually sluggish or practically non-motile and its small size, 6 to 12 microns, allows it to be passed by under the eye of a casual observer. When fresh and kept at body temperature it is more active. The ectoplasm and endoplasm are not sharply differentiated, the former sometimes showing as a clear hyaline rim in the rounded resting stage. Pseudopodia are ordinarily

small and sluggish. The endoplasm is granular, the nucleus within it usually not visible.

The cysts, measuring about 8 to 10 microns in length, are usually elongated or ovoid and one side of the rim may be less convex than the other. Seen fresh they are hyaline bodies without very definite

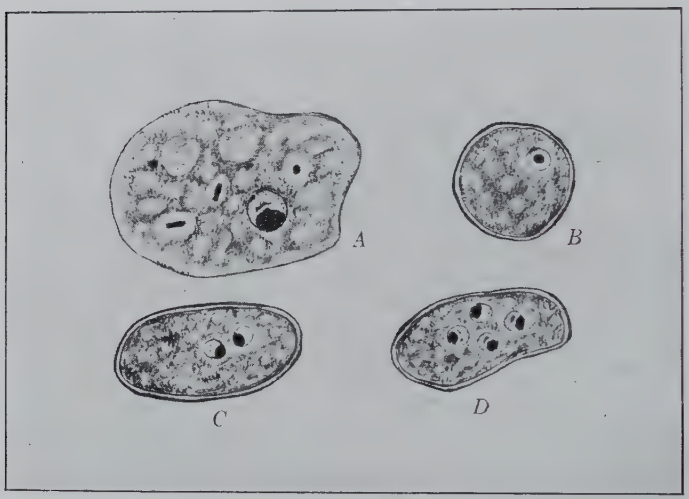


FIG. 20.—*Endolimax nana.*

Drawings of specimens stained by iron-haematoxylon. *A*, trophozoite. x 3500. *B*, *C* and *D*, cysts with one, two and four nuclei. x 1500.

wall, and the nuclei are invisible. Iodine preparations of fresh cysts may show the nuclei but not as clearly as in some other cysts.

For satisfactory study and identification of *E. nana*, it is virtually necessary to have well differentiated iron-haematoxylon preparations. In such

specimens both in active and encysted stages the nucleus may be correctly observed and upon this depends the identification of the organism.

The nucleus has an inconspicuous rim on which are no chromatin granules. This material is collected into the karyosome, which is a comparatively large irregular chromatin body or collection of granules, usually well to one side of the nucleus. This body may show a larger granule on one side and a smaller one on the other, connected by a thread. The cyst has four of these characteristic nuclei, commonly in one end of the cyst.

It may be readily appreciated that the vegetative form may be confused with small sluggish *Endamoeba histolytica* and that the cyst may also be falsely identified as the cyst of that important parasite. One should be warned, however, by the small size, the sluggishness of movement, the shape of the cysts, and the difficulty or impossibility of showing the nuclei even in iodine preparations. Positive identification rests upon stained preparations in which the nucleus can be accurately studied.

Endolimax nana inhabits the large intestine of man and its visible food bodies are bacteria. Wenyon (1920) saw it in the lumen of mucous glands in sections. The writer (1923) failed to find that it invaded ulcers of the rectum in a person who harbored it in the intestine. There is no indication that it ever causes harm to its host.

Thomson and Robertson (1925) maintained

growth on L. E. A. medium of Boeck and Drbohlav for nineteen days.

IODAMOEBA BÜTSCHLII

There is some confusion about an amoeba of the human intestine which is fairly commonly seen, the cysts of which were discovered before the active form

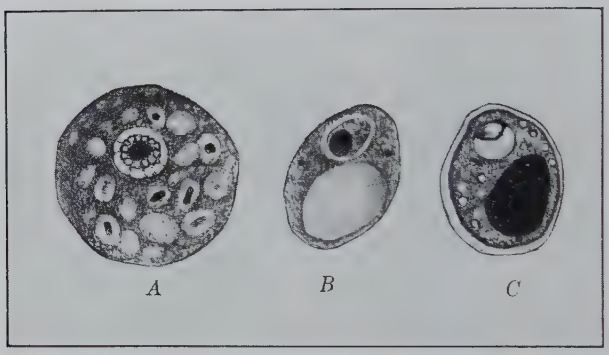


FIG. 21.—*Iodamoeba bütschlii.*

A, trophozoite, *B,* cyst stained by iron-haematoxylon, *C,* cyst with dark iodine stained globule. Drawings x 2000.

and called "iodine cysts" by Wenyon (1916). In the active stage this amoeba resembles somewhat *Endamoeba coli.* It usually measures 9 to 13 microns, is without distinct differentiation of ectoplasm from endoplasm, and is of sluggish movement. The nucleus is invisible in unstained preparations, as contrasted to *E. coli.*

The cysts, more commonly found than the vegetative form in stools, are of irregular shape, contain only

one nucleus, have a definite cyst wall, and characteristically show a more or less prominent refractive body staining dark brown in iodine, thus causing the designation of iodine cysts or "I cysts."

As in the case of *Endolimax nana*, carefully differentiated stained specimens of this amoeba are necessary for its identification.

The nucleus is single in all stages, except the rare occurrence of a bi-nucleated cyst. In the free stage this body shows a large central karyosome surrounded by globules between it and the rim. In the cyst the karyosome is on one side, the other being occupied by the globular bodies. The glycogen body is dissolved in the usual staining technique, leaving a vacuole.

Presumptive identification in practical stool diagnosis may be made on the following points:

An amoeba resembling *Endamoeba coli,* usually smaller, with no nucleus visible in the active form, with cysts commonly misshapen, showing in iodine preparation a large brown globule, and on careful examination a single nucleus in the cytoplasm not occupied by the glycogen.

This amoeba is also known as *Iodamoeba williamsi* and *Entamoeba nana.* It is a fairly common inhabitant of the intestine, where it is a harmless lumen dweller, its visible food objects being bacteria.

Thomson and Robertson (1925) report its cultivation on the L. E. A. medium of Boeck and Drbohlav for fifty-six days.

DIENTAMOEBA FRAGILIS

This is an uncommon amoeba of the human intestine, first described by Jepps and Dobell (1918). It is small, 3½ to 12 microns, actively motile when fresh and warm, with distinct ectoplasm and endoplasm, the pseudopodia being clear and "leaf-like."

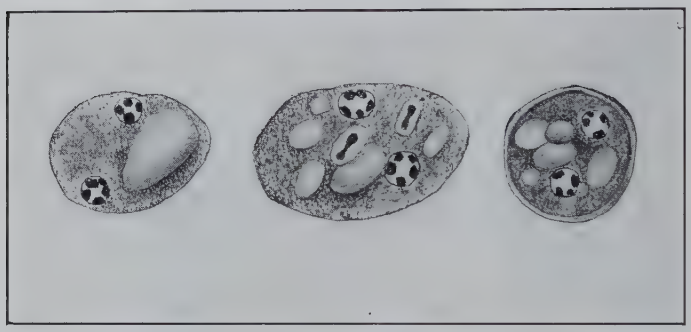


FIG. 22.—*Dientamoeba fragilis.*

Drawings of trophozoites and cyst from specimens stained by iron-haematoxylon. x 3000.

The endoplasm contains bacteria upon which the amoeba feeds.

Characteristic of it are its two nuclei, although these are usually invisible in unstained specimens. In properly stained preparations the nuclei show a rim with a scattering of small granules upon it, a central karyosome of a group of chromatin granules, connected with the granules on the rim by delicate threads.

Dientamoeba fragilis is an exceedingly delicate organism, undergoing early degeneration and disin-

tegration in stools. Its cysts are relatively unknown. Consequently it is to the ordinary examiner a rare or unknown amoeba. It is apparently harmless to its host.

Thomson and Robertson (1925) report its cultivation on Boeck and Drbohlav's L. E. A. medium.

OTHER AMOEBAE OF MAN

Karyamoebina falcata. Kofoid and Swezy (1924 and 1925) describe a new genus and species of amoeba from the intestine of man, occurring in six instances out of 13,894 stool examinations of 3000 persons. "This amoeba is characterized by one, rarely two or more, blunt hyaline pseudopodia." Ectoplasm and endoplasm are sharply separated. The endoplasm is coarsely vacuolated; food bodies are rare in these vacuoles.

"The nucleus contains one or two, rarely more, large crescentic, siderophile masses applied to the nuclear membrane and little chromatin otherwise applied to the membrane." There is a "neatly spherical, eccentric karyosome." Cysts were not found. The organism resisted treatment for intestinal amoebiasis.

Caudamoeba sinesis. Faust (1923) has described from cases of amoebic dysentery an amoeba, ingesting both red blood corpuscles and bacteria, and moving in a slug-like manner, having definite polarity, with the

nucleus in the forward endoplasm, and the posterior end dragging behind a mass of attached débris. No cysts were observed. Confirmatory evidence will be necessary to show the nature of this amoeba. It is not clear that it could not be *Endamoeba histolytica.*

CHAPTER VIII

The Flagellates

Classification. General Considerations. Prevalence and Relations. Examination for Flagellates.

CLASS: MASTIGOPHORA.
ORDER: MONADIDA.
GENUS: TRICHOMONAS.
SPECIES: *T. hominis.*
T. buccalis.
GENUS: CHILOMASTIX.
SPECIES: *Ch. mesnili.*
GENUS: GIARDIA.
SPECIES: *G. intestinalis.*
GENUS: EMBADOMONAS.
SPECIES: *E. intestinalis.*
GENUS: ENTEROMONAS.
SPECIES: *E. hominis.*
GENUS: TRICERCOMONAS.
SPECIES: *T. intestinalis.*

THE FLAGELLATES (FLAGELLATA)

Of the class of Protozoa known commonly as the Flagellata there are five well established genera occurring in the alimentary tract of man, there is a

sixth of probable validity and there may be others of very small size not yet sufficiently identified. The class has the common characteristic of possessing flagella, comparatively long lash-like organelles concerned with locomotion and food gathering. Their bodies are variable in a common "pear" shape.

Probably the majority of protozoologists most familiar with these organisms now believe the class to be harmless to their hosts, although some hold the question open. Among medical people there exists the greatest confusion as to the different genera and species and as to their effect on their hosts, a great many practitioners believing that at least some of them are disease producers. To these such terms as "flagellosis," flagellate infection, flagellate diarrhoea, and flagellate dysentery, relate to clinical states of more or less definiteness in their minds.

In many medical laboratories the only names known are "flagellate," *Trichomonas*, or *"Cercomonas,"* and the finding of such an organism in the stool is sufficient evidence for the institution of treatment of the conditions supposed to be due to it. The confusion is so great that it is necessary to discount or ignore the bulk of medical report on the subject.

There exists no reason why any examiner may not easily identify the common flagellates of the alimentary tract of man, *Trichomonas, Chilomastix*, and *Giardia*, although the very small ones, *Embadomonas, Tricercomonas*, and *Enteromonas*, require considerable acuteness.

The problem of the effects of these parasites upon man has reached a stage of *impasse*. None of the class has been shown to be an actual tissue invader or destroyer, none has been shown to produce any substance or bring about any state deleterious to the host. There is no positive direct evidence on the question, it is all clinical circumstantial evidence based upon the occurrence of indefinite symptoms of abnormal states associated with the presence of the parasites. Such evidence, if it were definite and characteristic, would weigh more than it does. There is no typical or characteristic clinical complex to be related to the presence of any of them.

Until better identification of the organisms and more careful relation of them to definite clinical states is done in medical practice the question will remain as it is, unless experimentation develops more bearing than it has.

It was thought that cultivation, which may now be done with all except *Giardia*, would initiate some direct avenue of approach, perhaps through isolation of the organisms and experimental infection. Thus far this has not occurred, cultures have not been developed which are usable in such work. Neither is animal experimentation satisfactory. It is most difficult to judge or control the absence of like parasites in experimental animals.

The species of flagellates found in the alimentary tract of man are apparently specific for him. We probably do not obtain these organisms from other

animals but from each other. They are transmitted from person to person more or less directly. Close personal contact, faulty personal toilet hygiene, unsanitary disposal of human excrement, contamination of water, milk, and food, exposure of excreta and food to flies, all are factors in the spread of these parasites.

Some of the species are very common, although varying in incidence in different quarters. All are of world-wide distribution. With the commonness of *Trichomonas, Chilomastix,* and *Giardia* in the general population of the world, it is readily appreciated that a certain percentage of the sick who come under the care of the medical profession will show one or more of them. It may be conservatively estimated that from twenty-five to fifty per cent of people in any region will be found to harbor an intestinal flagellate and that practically all people give tenantry to one or more at some time.

The fundamental fault, it seems, in most clinical studies of "flagellosis" is the lack of control study. It is recognized that proper scientific control of such studies, which do not lend themselves to experimental confirmation, is most difficult. Having in mind to compare a considerable group of flagellate hosts to a similar group of non-parasitized, the writer (1926) made an analysis of 1040 consecutive clinical cases in whom gastro-intestinal studies had a prominent place on account of the symptoms displayed, the study being spread over a period of about four years.

From only one stool examination, as a rule, of each, 23% of this number harbored flagellates as follows: *Trichomonas hominis* 12.5%, *Chilomastix mesnili* 7.5%, *Giardia intestinalis* 2.3%, *Tricercomonas* 1.5%, unidentified flagellates 1.7%.

In this series the incidence in the warm months was above the average (27%) and in the cold months below (15%), showing that, as a class, warm weather gives better opportunity for their spread and, also, that spontaneous elimination must take place.

City dwellers and those living in rural districts were similarly infected, 71% of the flagellate hosts and 70% of the free living in the city. From this observation it seems apparent that modern sanitary facilities, which existed in the places called cities here, do not prevent the spread of the flagellates. This has been observed by others, particularly in institutional surveys. Close personal touch, person to person transmission, seems to play a large part in their dissemination.

Males were slightly more commonly parasitized than females, 47% of the flagellate hosts and 40% of the free being males. Smithies (1926) has made a similar observation. Of his 265 patients harboring intestinal protozoa 172 were males. Perhaps men, by coming in contact with more people, come more into contact with the flagellates.

Forty-three per cent. of the flagellate infested patients were below forty years of age and 68% were below fifty, while in the flagellate-free group 53%

were below forty and 83% were below fifty. The later years of life increase the liability, perhaps from cumulative contact and infection, perhaps in relation to certain other states which are more concerned with later life.

One of these possibly related states is that of relative or actual gastric anacidity. Thirty-two per cent. of the parasitized patients showed no free hydrochloric acid, while this condition was present in but 19% of the flagellate-free. On the other hand, 25% of the flagellate-free showed hyperchlorhydria, while this was the case in but 10% of the flagellate hosts. The remainder, 57% of the parasitized and 56% of the free, in round numbers, were in the normal region, counting broadly any acid up to fifty degrees as "normal." Does this more frequent finding of hyperchlorhydria in the flagellate-free and of achlorhydria in the parasitized mean that flagellates are instrumental in pathologic states which lead to decrease in acid secretion, or does lowering of the acid barrier in the stomach explain the higher incidence of the flagellates? Experimentally *Trichomonas hominis* had a few survivors in 0.1% hydrochloric acid in 0.9% sodium chloride solution at the end of two hours incubation. It was entirely destroyed within this time by stronger acid solution. *Chilomastix mesnili* survived acid exposure even better than did *T. hominis*, being still active at one and one-half hours in 0.1% and 0.2% HCl in 0.9% NaCl solution, but 0.3% acid killed within one half hour. *Giardia intestinalis*

trophozoites failed to survive a similar exposure to 0.1% acid for even a few minutes, although the writer has seen it active in gastric content in a state of achlorhydria. Of course, *Chilomastix mesnili* and *Giardia intestinalis* have encysted phases in which they must be more resistant, but it is not unlikely that *C. mesnili* in active stage may pass the stomach as well as does *Trichomonas hominis*, although the former is killed rapidly by water alone.

Gastric and duodenal ulcers are conditions in which hyperchlorhydria is common. They are also related to early life as contrasted to chronic cholecystitis, for instance. There was a clinical diagnosis of peptic ulcer in 10% of the flagellate-free but none in the others.

On the other hand, there was recorded a clinical opinion of chronic cholecystitis, some proven, the majority not, in 38% of the parasitized and 22% of the free. Chronic cholecystitis is a state of disease of later life and is commonly accompanied by lowered gastric acidity, both of which states were shown in definite relation to high flagellate incidence. Chronic cholecystitis is one of the conditions in which a tendency has been noted to incriminate intestinal protozoa and, considered separately, these observations could be taken as evidence in favor of such a view. As against such reasoning, however, one has to account for 22% of the flagellate-free who had the same diagnosis. In a debate one could say that it would be equally reasonable to so explain the higher in-

cidence of arterial disease occurring in the flagellate hosts.

A condition of chronic appendicitis—thought by some physicians to be sometimes produced by intestinal protozoa—was diagnosed in 22% of the flagellate infested and in 25% of the free.

Diarrhoea, the condition longest and most commonly ascribed to the flagellates, was present at the time of examination or conspicuous in the past history of 9% of the parasitized and 8% of the free. When diarrhoea was present there was no gross or microscopic characteristic feature. It was impossible to say that a watery stool or a frothy gassy stool was distinctive of the presence of flagellates. Constipation was much more prominent but of practically the same proportion in both groups, 57% of the parasitized and 55% of the free.

The benzidine test for chemical blood in the stool was positive in 67% of the flagellate hosts and 71% of the others, this observation being considered only of comparative significance. Similarly to be used is a clinical opinion of colitis in 6% of the parasitized and 11% of the free.

Blood counts and haemoglobin estimations revealed no apparent differences between the two groups, and nothing could be made of any attempt to relate various nervous system disturbances, which may be indicated by the term "neurasthenia," to the parasitized group more than the other.

Arthritis deformans, which Barrow (1924) found

in 11% of his protozoa infested cases and in which disease he found 100% intestinal protozoa infection, was conspicuous by its absence, there being no recorded case in the parasitized group. The search for this disease in the records of the flagellate hosts was done as an afterthought, the records of the others were not examined with this in view.

It will be seen that in such a study there is little or nothing to produce as evidence in favor of harmful effect of flagellates upon their hosts, but rather to the contrary.

EXAMINATION FOR FLAGELLATES

In examining for flagellates in feces it is well to have a procedure which fits with that of search for amoebae. It is the practice of the writer to ask for a fresh stool produced by a saline purgative, unless there is diarrhoea. As contrasted to the amoebae it is the active form, the trophozoite, which is most practically discoverable with the flagellates. Unless the stool is really fresh and liquid, trophozoites which are present may not be seen or at least not so readily. Numerous surveys giving a low incidence to *Trichomonas* were made on stools which were not fresh.

Mixed on a microscopic slide with 0.9% sodium chloride solution to a thinness so that, when covered, vision is clear, and examined with a low dry objective, feces will ordinarily reveal the active or-

ganisms immediately when considerable numbers are present. Prolonged examination of several preparations will usually reveal sparse infection. When picked up by low magnification they may be then studied under higher power objectives.

Mixing a portion of the specimen with 0.9% sodium chloride solution and placing in the incubator for a short time, until well warmed, will activate organisms which have become sluggish and will reveal parasites otherwise overlooked.

It is seldom that the trophozoite of a flagellate does not appear in a liquid stool examined properly immediately after passage or after being kept warm for a short time, when it is present in the intestine. For that reason the cysts are relatively unimportant from the standpoint of such an examination. They are more important in survey work, when large numbers of specimens are to be examined, or when examination cannot be done immediately. Such examinations are likely to miss *Trichomonas* and other flagellates when they are present only in the active stage.

The active forms of the flagellates are readily identifiable and there is little or no excuse for confusion of them, except some of the more minute uncommon species. Even in the most actively motile state, one learns to recognize *Trichomonas, Chilomastix* and *Giardia* by their manner of movement. *Giardia* has an easily recognized shape. Seen anteroposteriorly it is of pear shape with broad anterior

and tapering posterior extremities. A lateral view shows the broad concave indentation anteriorly and ventrally. It swims with a peculiar vibratory motion of the body and frequently flexes the tail to the anterior end. *Chilomastix* has a stiff inflexible body of the shape of a carrot. It swims in a characteristic "corkscrew" forward. *Trichomonas* has a typical darting motion, moving very rapidly when most active, frequently stopping and anchoring by its posterior end to a clump of débris, while it whips the solid particles about with its flagella in violent manner. It has a very flexible plastic body, twisting and turning, crawling among masses of solid particles. The undulation of its surface "ruffle" is most characteristic. When specimens have been exposed for a time and become sluggish, *Trichomonas* develops an undulating movement of the surface of the body, probably a slow movement of a relaxed undulating membrane, and the flagella may move so slowly that they can be readily seen or even counted. In such a state *Giardia* and *Chilomastix* remain rigid, their form and shape does not change, their flagella gradually cease motion, hanging as idle threads, while the undulating flagellum within the ventral groove may continue a sinuous motion.

Most painstaking study and carefully stained specimens are necessary for identification of the uncommon more minute species, *Embadomonas intestinalis, Tricercomonas intestinalis* and *Enteromonas hominis.*

The cysts of *Giardia intestinalis* and *Chilomastix mesnili* may show in diarrhoeal or purged stool or they may occur only in a natural formed stool. *Giardia* cysts are commonly numerous; they are ovoid, of ground glass appearance, reveal their inner structure in iodine preparation, and are readily discoverable. A small ovoid or slightly elongated "ground glass" body which shows "eyes" (the nuclei) in one end on careful observation, is likely to be a *Giardia* cyst.

Cysts of *Chilomastix mesnili* are not nearly so readily found. In diarrhoeal stool or purged specimen the trophozoite may be the only form. When present in a natural stool their number is variable and prolonged search may be necessary to find them. They are smaller than *Giardia* cysts, are typically pear or lemon shaped, are of uniform size, "ground glass" appearance, and show in iodine preparation a dot on one side anteriorly (the nucleus). They are apt to be confused with yeasts by the inexperienced.

Specimens stained by the iron-haematoxylon method are necessary in case stools are not to be examined in a reasonable period of time. Stained specimens are essential for study of the finer details of the organisms, the flagella, the undulating membrane, nucleus, food bodies, etc. For the inexperienced such preparations are necessary for the identification of the cysts. In carefully prepared specimens staining by the ordinary blood film methods, such as Wright's or Leishman's, may give beautiful demon-

stration of the flagella, undulating membrane, the cytoplasm and its food bodies, but these stains do not give nuclear detail and are generally of little or no service in differentiating protozoa.

A little careful study and practice will enable the laboratorian to dispense with time consuming and difficult staining methods in routine examination for flagellates in feces. Similar fresh unstained preparations from the gums and vaginal vault are satisfactory for discovery of *Trichomonas* in these locations. In urine the ordinary microscopic examination of centrifugalized specimens will reveal *Trichomonas* when present. One must, however, be continually on guard against free living forms which may come from water in specimen bottles or from solutions used in the examinations or may grow in specimens allowed to stand. Fresh specimens taken in dry clean containers are essential to avoid confusion and even then one will occasionally encounter a free living flagellate. Check of the water and solutions used about the laboratory will commonly reveal the source of a perplexing flagellate.

Hegner and Becker (1922) and Hill (1926) have recommended the culture method as a practical measure for routine diagnosis or field surveys. The need for such methods in medical practice is not impressive; the greater need is for careful routine practices in stool examination and careful identification of the discovered organism. Culture methods, even yet, are crude at best, and they are not easy of application.

Giardia has not been cultivated and would not be revealed in such studies. Few, if any, intestinal protozoa will be found by the average laboratorian by culture when not by measures here outlined, if properly carried out. Later, perhaps, but now we are interested in avoiding further confusion and of clearing some which exists.

CHAPTER IX

The Flagellates (Continued)

Trichomonas Species. Cultivation. *Trichomonas hominis.* Prevalence and Relations. Habitat and Effects. Treatment. Other Intestinal Trichomonads.

TRICHOMONAS

When considered by genera *Trichomonas* is the most common flagellate of man's alimentary tract, in the writer's experience. *Trichomonas hominis* is the inhabitant of the intestine while *Trichomonas buccalis* occurs in the mouth.

It is not yet clear that these two are distinct species, or what their relation is to *Trichomonas vaginalis,* the habitat of which is the vagina. In their own natural locations these three trichomonads of man may have somewhat different appearances. *Trichomonas vaginalis* may be larger, and it and *Trichomonas buccalis* usually swim about less actively than does *Trichomonas hominis,* as it is seen in liquid feces. When seen in culture, however, it is thus far commonly impossible to say from which source the organism came.

In fact, Hegner (1928), by experimentally estab-

lishing infection of fowls with trichomonads from seven different species of animals, raises the question whether these may not be a single species and not separate as now generally regarded.

The same investigator (1928) by producing infection of the vagina of monkeys with monkey trichomonads of intestinal and vaginal origin, brings this experimental evidence in support of the morphological resemblance between the parasites of these two locations, to which he and Ratcliffe (1927) had previously referred.

The present writer (1915) reported upon *Trichomonas* from the vagina and mouth of the same individual, which was morphologically and culturally the same organism and was, therefore, designated as *Trichomonas vaginalis,* that being the acceptable name in that case. Later Ohira and Noguchi (1917) cultivated the mouth trichomonad and, considering it distinct from *Trichomonas vaginalis,* called it *Tetratrichomonas hominis,* a name not available since the organism is identified as belonging to the genus *Trichomonas,* which, with *Trichomonas vaginalis* as the type species, has four flagella, not three as assumed by these writers, and since the specific name *hominis* was already applied to the species in the intestine.

Goodey (1917), a bit later, named the mouth trichomonad *Tetratrichomonas buccalis,* but since *Tetratrichomonas* is a synonym to *Trichomonas* their corrected name is *Trichomonas buccalis.*

The writer (1922) called attention to Steinberg's

(1862) three names, *Trichomonas elongata, Trichomonas caudata,* and *Trichomonas flagellata,* for *Trichomonas* of the mouth, the first of which, by priority, should be the valid name, although Kofoid's (1920) acceptance of *Trichomonas buccalis* was taken as expedient. Wenyon (1926) adopts *Trichomonas elongata* as the correct name for *Trichomonas* of the human mouth.

Lynch (1922) cites the morphological resemblance of the trichomonads of Ohira and Noguchi and of Goodey with *Trichomonas* from the vagina and mouth in his studies and is of the opinion that they are the same organism. It is further called to attention (Lynch, 1922) that trichomonads may vary in activity and in size in different environments and that differentiation in the same medium would seem necessary to prove the validity of different species from different sources unless there may be some distinct feature of difference not now known.

It seems, therefore, not impossible that *Trichomonas* of man is all one organism instead of three. Further, it seems that the habit of differentiating and naming protozoa by location is unsound and that distinction among them should rest upon features of difference between the organisms themselves. However, it is conventional at the present to consider *Trichomonas hominis, Trichomonas elongata (buccalis),* and *Trichomonas vaginalis* as distinct species and this will be adhered to in this writing.

Trichomonas has been the subject of much investi-

gation and experimentation of recent years. The author (Lynch, 1915A and 1915B), Ohira and Noguchi (1917), Boyd (1918), Pringault (1921), Hogue (1921) and, more recently, numerous others have cultivated it from various sources. Excellent results have been obtained with the organism from the mouth, vagina, intestine, and from urine *(Trichomonas vaginalis)*, in liquid human blood serum diluted with from four to ten parts of 0.5% to 0.9% sodium chloride solution, Ringer's solution, and Locke's solution, incubated at 37.5°C. The higher dilution has been attended with more success in the case of the intestinal trichomonad, the stronger solution with that from the mouth. Those who are interested in research in culture methods should consult the original articles of those who have reported upon it.

Ability to produce prolifically varies in different cases, and probably the associated bacterial growth has much to do with this. All of these media are slightly alkaline and usually become more so with the age of the culture. The column of medium should be rather deep, and the organisms may be taken from the bottom of the tube with a capillary pipette. The numbers are usually greatest at about the third or fourth day but some may live for twelve days or longer. Subculture should be planted on the second or third day.

Hinshaw (1927) gives the simplest medium he has used as composed of 100 cc. of Locke's solution with

10 cc. of 10% casein, tryptic digest, while the medium of most prolific cultures was composed of the solidified egg slant of Boeck and Drbohlav, given in the cultivation of *Endamoeba histolytica,* covered by a liquid composed of Locke's solution 1000 cc., with the white of one egg and rabbit blood serum, one drop to each 2 cc. The blood serum was added aseptically to each tube, introduced at the base of the slant just before inoculation of the culture. This investigator believes that the split products of protein are essential in the cultures and that bacteria are necessary for the purpose, he having failed to grow *Trichomonas* in the absence of bacteria. On the other hand, some bacteria were definitely harmful to the growth of the protozoa.

Cultivation of *Trichomonas,* in the writer's experience, while subject to success in considerable proportion and in a variety of media in which a protein in solution in a physiological salt solution are the essentials, is in a measure uncontrollable. We do not know, except in a very rough fashion, what the essential elements are. When we do we shall be able to cultivate it without bacteria. The culture method does not seem available for general practical use at the present. Most of the methods described as successful will fail at times under exact imitation, so far as is possible, of other occasions when they succeed.

TRICHOMONAS HOMINIS

The trichomonad of the human intestine, *Trichomonas hominis,* is apparently variable in its prevalence in different localities, at least so in the experience and reports of reliable students of it.

Craig (1926), from an analysis of several studies by Kofoid, Kornhauser and Plate in the United States, Mathews and Smith and Jepps in England, Faust in China, and from his own observations, is of the opinion that this organism is the least common of the better known intestinal flagellates. He believes that it is present in only from 0.5% to 1% of the inhabitants of temperate regions.

Compiling the results of a number of reports published by English, American and French investigators from 1916 to 1919, Hegner and Payne (1921) arrive at an average incidence, in 20,000 reported cases, of 3% for *Trichomonas hominis.*

Boeck and Stiles (1923) give an incidence, in 8029 persons examined by them, of 0.007%. However, the conditions of their examinations did not favor trophozoites and probably this organism was largely missed.

Jepps (1923), examining 674 Tamils and 350 Chinese in the same hospital, found *Trichomonas hominis* in 16.5% of the former and in 2.2% of the latter.

Barrow (1924) found less than 2% in 725 of his patients harboring some type of intestinal protozoon. He found an incidence of 8.3% in 79 cases of arthritis deformans.

Smithies (1926) found 2% of 3780 patients having some gastro-intestinal disturbance to harbor *Trichomonas hominis.*

Thomas and Baumgartner (1925) found only 0.26% infestation of 1122 inmates of a New York state school for feeble-minded women.

Hegner (1925) found 20.6% of 286 patients in hospitals of Central America to harbor the parasite, this from only one examination.

Hill and Hill (1927) found it in 20.9% of children of pre-school age in Porto Rico.

The writer (1926) found 12.5% of 1040 cases of chronic disease having some reference to the gastro-intestinal tract to show *Trichomonas hominis* on about one examination per person. It is believed that more than 10%, probably nearer 20%, of the inhabitants of warm countries will show *Trichomonas hominis* on correct examination.

It seems probable (Lynch, 1928) that *Trichomonas* infestation of the intestine is more related to rural life and to warmer climates. This opinion, deduced from comparative studies, is consistent with the life of the organism. It is a naked, non-encysting flagellate of remarkable ability to survive exposure.

On account of its non-encystment it is very susceptible to drought.

Hegner (1928) finds that a temperature of 41°F. (5°C.) does not effect the viability of the organism beyond that of room temperature (70°F.), that feces deposited in wet garden soil retained viable organ-

isms for as long as seven days, but that a sandy soil bed led to disappearance by culture after twelve hours. He found the viability of the organism in water to vary with dilution, such dilution as would occur when a stool is washed into a pond or stream giving little or no opportunity for survival and transmission of the organisms. This is an interesting observation, since it has a direct bearing on the means of transmission, commonly believed to be important. Others have found *Trichomonas* resistant to water. The writer's experiments on the point are contradictory and indicate an uncontrolled factor. At one time neither tap water or distilled water effected *Trichomonas hominis* in a test tube in the incubator for about three hours. At another time tap water killed within two hours. At still other times, controls for cultures, in tap water, incubated overnight, failed to survive, but a similar failure has occurred in physiological saline solutions. The vomitus and feces of flies fed on feces containing *Trichomonas hominis* produced viable organisms at various intervals between twenty minutes and six hours after ingestion. Cockroaches were not found to pass viable organisms after ingesting them. *Trichomonas hominis* in feces was destroyed by 2% carbolic acid in 5 minutes, 1% creolin in 5 minutes, and 0.5% lysol in 5 minutes.

Trichomonas hominis is a pearshaped flagellate measuring from 5 to 15 microns in length. When very active it darts about in quick motion, swimming

freely in liquid, revolving as it goes, crawling, twisting and pushing its way through clumps of débris. Its body is flexible and it may elongate, contract and turn at will. When sluggish it may have surface pseudopodia. It has four flagella attached at the anterior end to a group of granules, the blepharoplast. It possesses an undulating membrane, the border of which (axoneme) originates at the same

FIG. 23.—*Trichomonas hominis.*

Drawings of specimen unstained (A) and stained (B) by iron-haematoxylon. x 2000.

place as the flagella and may extend posteriorly as a free flagellum. The base of the undulating membrane is supported by a fibre of similar origin. The flagella move in rythmic lashings, sweeping materials against the ingesting surface, the undulating membrane waving synchronously with the lashing of the flagella. The undulating membrane extends diagonally posteriorly for the whole or greater part

of the length of the body. A skeletal or stiffening structure, the axostyle, extends from the region of the nucleus through the length of the body and projects as a sharp spine from the surface near the posterior end. There is an indistinct slit or cytostome at the anterior end near the origin of the flagella opposite to the undulating membrane.

The nucleus is near the base of the flagella and undulating membrane, is invisible in ordinary active unstained specimens, and its detail is not easily studied even in stained specimens. It is ovoid and has a definite membrane. The distribution of chromatin is not well described, the karyosome is considered to be central.

The division of the organism is by binary fission, the nucleus undergoing mitosis, according to Kofoid (1915).

In division the body increases in size, it becoming increased laterally more than longitudinally. The nucleus divides and probably the axostyle and the undulating membrane split longitudinally from before backward. The flagella apparently do not split but are evenly divided between the two new organisms. The appearance of the dividing organism becomes shield shaped with an indentation between the two new anterior poles, which may have two flagella each. The undulating membrane may be separated anteriorly and joined posteriorly. Anterior schism proceeds until the organism is completely divided, the posterior extremities adhering

for a time before the organisms swim separately. By this time the complement of organelles of each is usually complete. Such division may be watched in cover glass preparations from cultures.

If *Trichomonas hominis* ever encysts it is unknown. The writer (1915 and 1916) erroneously described two cysts for the organism, the first was *Blastocystis*, which had been so described by Ucke (1908), by Bohne and Prowazek (1908), and Bensen (1901). The second, an original error, was the cyst of *Chilomastix mesnili*, not well known at that time.

HABITAT AND EFFECTS

The natural habitat of the intestinal *Trichomonas* is the colon, particularly the caecum.

After ingestion it passes through the stomach and small intestine but probably does not become seated until it reaches the caecum.

It is apparently solely a lumen dweller. Wenyon (1926) found it once in the lumen of the glands of the large intestine and passing from the glands into the interstitial tissue. He states that the intestinal *Trichomonas* of the guinea pig, *Trichomonas caviae*, often invades the wall of the large intestine, which shows ulceration, in that animal. Wenyon thinks that tissue invasion may occur and cites the observations of Pentimalli (1923), who found the organism in the blood, and Kessel (1925), who saw it in the pus of an amoebic liver abscess, but does not know

that this is an indication of pathogenicity. Hogue (1928) has grown *Trichomonas* in tissue cultures of epithelium, fibroblasts and sympathetic nerve fibers without apparent injury to these cells.

The work of Hadley (1916), who thought the tissue invading parasite of turkeys in the disease Blackhead was a *Trichomonas,* has not been supported.

Experimental infection, such as recorded by the writer (1915), has not shown the production of disease in a convincing way. Such experiments are subject to a variety of error.

The occasional observation of a red blood corpuscle ingesting trichomonad cannot be held to indicate any pathogenic rôle according to Hegner (1928), who induced eight species of *Trichomonas* from as many different animals to ingest red blood corpuscles from seven different species of animals, in culture tube.

An experiment of the writer in which *Trichomonas hominis,* as well as *Blastocystis,* was cultivated in a medium composed of one part of the host's own blood serum to nine parts of 0.9% sodium chloride solution, suggests that there are no inhibitory or destructive antibodies produced by the host and, therefore, probably no toxic absorption.

The condition which most often calls attention to its presence and in which it is most often suspected of an etiologic rôle is diarrhoea, with frequent watery stools of no peculiar gross or microscopic character.

Here it must be kept in mind that, in case parasitism exists, diarrhoea would bring the organism in activity and numbers not ever seen in the natural stool. It must, also, be recalled that an intestinal state in which there is inflammatory exudation, with liquid content throughout its course, would furnish most excellent opportunity for multiplication of the organism and it would naturally tend to appear in the liquid stool in unusual numbers. It would furnish excellent conditions for cultivation. The administration of a purgative may bring out tremendous numbers when they may be scant or unobserved in the natural stool.

The common vague clinical state, in which there is nervous debility, deficient appetite, "indigestion," with occasional attacks of diarrhoea in a chronic state of constipation, flatulence and gaseous eructations, abdominal discomfort of indefinite kind, and various systemic dysfunctions, which is at times laid at the door of *Trichomonas hominis,* is such a common state, regardless of *Trichomonas,* that it cannot be said at the present whether it may be ever due to this parasite.

The writer (1928) found that it had no particular relation to any age period, 53% of *Trichomonas* hosts being below 40 years of age and 78% below 50, while of the flagellate-free 53% were below 40 and 83% below 50. Children and the aged were found infected.

Nineteen per cent. of the flagellate-free group of

this study, and 23% of those who harbored *Trichomonas hominis* showed a condition of gastric anacidity. Twenty-five per cent. of the flagellate-free and but 14% of the carriers of *Trichomonas hominis* had gastric hyperacidity. Experimentally *Trichomonas hominis* is mainly destroyed in 0.1% HCl in 0.9% sodium chloride solution in two hours, and all are killed by stronger acid mixture.

There was a history of diarrhoea in 8% of the free and 6% of the parasitized, while 55% of the former and 50% of the latter were habitually constipated.

There was no change of the blood or other abnormality which was not equally conspicuous in the two groups, except that the records of the hosts of *Trichomonas hominis* showed a clinical diagnosis of chronic cholecystitis in 44% as against a like diagnosis in 22% of the controls.

Those who are convicted of the pathogenicity of the parasite will consider this as distinct evidence in favor of that belief, while those who are opposed will ascribe it to the creation in this clinical state of conditions more favorable to the seating and survival of the parasite in the intestine, particularly in favor of it being able to pass the stomach. It must be borne in mind that a clinical diagnosis of chronic cholecystitis is itself an indefinite thing.

The writer has used the term "Trichomoniasis" as relating to the activities of *Trichomonas* in man, at that time believing it to be definitely a disease producer. More mature observation, however, and

analysis of the work of others, forces one to reasonably retain an open mind. There exists at the present no definite evidence of the pathogenicity of this organism; most positive evidence is opposed. On the other hand, however, there exists no definite proof that it may not cause harm to its host. Trichomoniasis, then, means at the present only the state of carrying *Trichomonas*. Dobell (1921) is horrified by the term. Perhaps it was a premature designation of a state not sufficiently proven, but if it is never proven as a disease state the term will never be used or needed.

TREATMENT

To speak of treatment, either preventive or curative, of an infection, when obviously it is not known whether it should be treated, is also premature.

Prevention of the spread of the parasite apparently needs particularly the proper disposal of human excreta, the prevention of its use as fertilizer for garden stuff, sterilization by some such agent as 0.5 to 1% lysol in case of lack of proper sewage disposal, the control of flies, the protection of excreta and food from them, and more careful personal hygiene. The fly and the human being of fly habits are the sources of spread.

The writer knows of no specific treatment which may be depended upon to eliminate *Trichomonas hominis* from the intestine.

Various drugs have from time to time been claimed

to destroy it. Critical examination disproves these claims, largely or wholly.

Emetine, mercury, salvarsan and various arsenicals, methylene blue, and others have been recommended.

The claims of those who recommend the various intravenous arsenicals seem to need substantiation, since a number of people who have had extensive courses of antisyphilitic treatment by these drugs harbor the organisms. Neoarsphenamine in dilution of 0.45 gms. in 100,000 up to 0.45 gms. in 1000 in 0.9% sodium chloride solution has no effect on the motility of *Trichomonas hominis* in one hour and in the higher dilution it survives and is active at twenty-four hours. Neosalvarsan, as strong as 0.45 gms. in 1000 cc. of 0.9% sodium chloride solution, has no appreciable effect at one and one-half hours. Sulpharsphenamine in similar mixture has no effect at one hour. The latter apparently effects the organism by direct application before the former does.

It must be recalled that the number of trichomonads in the intestine and in the stool varies widely without therapeutic influence and that the finding of a few organisms or none at an examination or on repeated examination does not necessarily indicate that any therapeutic agent was responsible.

While *Trichomonas hominis* is at times a persistent parasite and may remain for long periods, it undoubtedly spontaneously dies out. Otherwise the increasing years would show higher incidence, and

we would all probably come to be hosts before reaching old age.

Diet and the intestinal flora may have much to do not only with the number of the parasites in the intestine but with its gaining foothold and its survival. Hegner (1923) found that a vegetable diet would increase and a carniverous diet would decrease the flagellates in rats. Ratcliffe (1928) found that this effect of high protein diets is for the most part due to the influence on the multiplication of proteolytic anaerobes in the rat's intestine. The most effective protein was casein, and beefsteak to a lesser degree.

An exclusive or largely milk diet, either whole milk or bacillus acidophilus milk cultures, is without effect and the latter may be contraindicated.

It would seem that intravenous medication is not to be recommended except, possibly, as an experimental measure.

It would be well to give trial to such drugs as stovarsol, which may be administered by mouth, accompanied by a diet largely or almost exclusively of such meat as beef muscle or at least of high protein content, for a period calculated to develop a high content of proteolytic flora in the intestine.

Escomel (1913 and 1917) claims to cure infection of intestine or vagina with *Trichomonas* by the use of turpentine, in the intestinal infection using the drug by mouth and by enema. He gives a tablespoonful of the following mixture every two hours for three days: oil of turpentine, 2–4 gms., camphorated tinc-

ture of opium, 6–12 gms., acacia, 120 gms., syrup acacia, 30 gms. During this time the diet is restricted to carbohydrates and rice water. At the same time he gives from one to three retention enemas of 15–30 drops of oil of turpentine, with 10 drops of tincture of opium, and a beaten egg yolk, in 4 tablespoonfuls of boiled water. This therapeutic enema follows an evacuating injection.

OTHER INTESTINAL TRICHOMONADS.

Derrieu and Raynaud (1914), Chatterjee (1915), Haughwout and deLeon (1919), and Kofoid and Swezy (1924) have called particular attention to a subgenus of *Trichomonas* carrying five anterior flagella instead of four. From time to time trichomonads of man have been described without the regulation number of flagella. Kofoid and Swezy (1923) name the five flagellated organism, *Pentatrichomonas,* the specific name for that of man being *P. ardin delteili,* and the three flagellated form *Tritrichomonas.*

Pentatrichomonas in man is given special significance by some of these writers on account of finding it in cases of dysentery and also its habit of ingesting red blood corpuscles. Kofoid and Swezy have cultivated the organism and determined its survival in rain, tap and creek water for three days.

CHAPTER X

The Flagellates (Continued)

Trichomonas elongata. Relations. Morphology. Habitat and Effects. Treatment. *Trichomonas vaginalis.* Prevalence. Transmission, Habitat and Effects. Treatment.

TRICHOMONAS ELONGATA (BUCCALIS)

As previously discussed, the *Trichomonas* of the mouth of man was given three names by Steinberg (1862), *Trichomonas elongata, Trichomonas caudata, Trichomonas flagellata,* he thinking he observed three varieties. The writer (1922) called attention to the priority of Steinberg's naming over that of Goodey, *Tetratrichomonas buccalis,* although conforming to Kofoid's acceptance of the corrected name, *Trichomonas buccalis* (Goodey). This was because *Trichomonas buccalis* had come into common usage. Following the example of Wenyon (1926), however, it is concluded that the first name used by Steinberg, *Trichomonas elongata,* is the correct one.

Trichomonas elongata has not been, with certainty, differentiated from *Trichomonas vaginalis* or *Trichomonas hominis* and should it be shown to be

identical with either or both, its name will be supplanted.

The writer (1915) studied trichomonads in the vagina and mouth of the same woman and is of the opinion that even if *Trichomonas elongata (buccalis)* is a valid species, *Trichomonas vaginalis* may occur in the mouth. In direct preparation and in culture the organisms from the two sources were indistinguishable.

Wenyon observed the organism in pus from the tonsil and it has been seen in sputum, supposed to have come from the lung, and in gangrenous abscesses of the lung at autopsy.

The writer (1915 and 1922), Ohira and Noguchi (1917), Hogue (1926), Hinshaw (1926–1927), and others have cultivated this organism in a variety of media, particularly in blood serum diluted with physiological saline solutions in deep columns of the tubed medium or as supernatent fluid on the egg slant of Boeck and Drbohlav, as given in the cultivation of *Endamoeba histolytica.*

Trichomonas elongata (buccalis) was found by Hinshaw (1926) in 57 of 186 persons, all having pyorrhoea. He believes that it is very common in advanced pyorrhoea but not present in normal mouths, and that culture methods are necessary to reveal its presence in many instances. Hogue (1926) found it in 9 of 50 persons selected at random, all by smear preparation, and all hosts being the subjects of diseased gums or teeth. Six of twenty-nine dogs bore

the infection, according to Kofoid (1929). Hegner and Ratcliffe (1927) found 22 of 23 dogs harbored a similar organism.

The form of *Trichomonas elongata* is the characteristic pear shape, being broader and more blunt anteriorly and tapering posteriorly. It is usually from 10 to 18 microns long by about 5 to 8 in width,

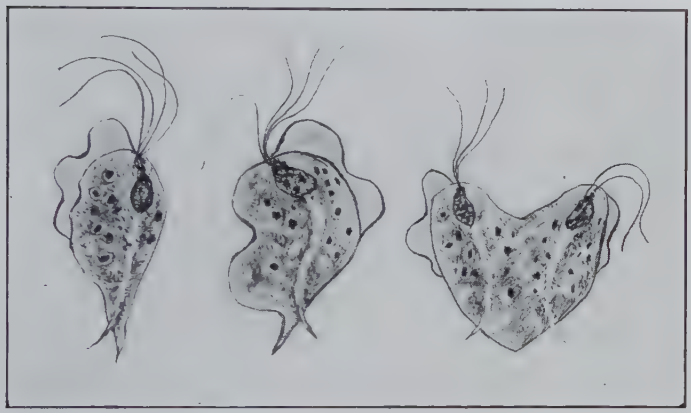


FIG. 24.—*Trichomonas elongata.*

Drawings of specimens from culture, stained by iron-haematoxylon. x 1800.

but much larger forms may be seen, especially in culture.

Its activity depends upon the material and environment of its growth. In heavy débris with little fluid it may crawl about, twisting and turning, elongating and shortening at will as it passes through the avenues of its course. Its body is flexible and plastic. In liquid medium as in fresh culture, or

from an exudate of an active gingivitis, it is exceedingly active, swimming dartingly about, with flagella and undulating membrane at high speed, twisting and turning, or becoming anchored caudally for a moment, while its peripheral organelles lash and undulate, threshing solid particles about in a violent fashion, then pulling loose from an attenuated anchoring appendage and swimming rapidly on its way. Nothing more than its darting motion and its possession of flagella and undulating membrane may be made out of active specimens. As it slows down from cooling or exposure the "whip-cracking" rythmic lashing of flagella synchronous with the undulating membrane waves, the propulsion of bacteria against the ingesting surface, the flexibility of the body, the axostyle, and the revolving of the body in forward motion may be seen.

It has four anterior flagella, which with the free margin and the basal fiber of the undulating membrane, and the stiff, posteriorly protruding axostyle, originate from the blepharoplast, within a knobbed anterior pole, which is the base of movement of the motor organelles. The posterior and inner part of the body may be seen to be vacuolated and to contain granules, bacteria. The nucleus is not seen in the unstained specimen.

Staining by Heidenhain's iron-haematoxylon method brings out the nucleus and the structural detail. Very pretty specimens may be made with Wright's or Leishman's blood film stains, by care-

fully developed technique, showing the several structures but no particular nuclear detail. The detail of structure may be found recorded by Hinshaw (1926).

As with other trichomonads of man, no cyst is known.

To find the organism it is necessary to take material from beneath the gums or between the teeth. This may be mixed on a microscopic slide with 0.9% sodium chloride solution to a dilution suitable for good vision when examined under a cover glass. The low dry microscopic lens reveals its presence and it may be studied under higher powers.

Dark field examination makes a conspicuous object of the organism, its flagella, undulating membrane, blepharoplast, and food bodies standing out as brilliant objects. When it slows down the flagella may be counted by this means, or by ordinary high power examination when they have become very slow or have ceased motion.

For the best stained specimens thin smears of the exudate of its habitat are made and stained as described under examination for amoebae.

Hinshaw (1928) produced experimental infection of the mouths of dogs with pyorrhoea.

Hogue (1926) found the thermal death point to be 45°C.

Hinshaw (1927) found it survived suspended in distilled water for 10 to 12 hours.

HABITAT AND EFFECTS

Transmitted, no doubt, in the active stage by practically direct passage from person to person, such as by kissing, possibly by the use of common drinking cups, and the placing in the mouth of objects from the mouth of another, the organism becomes seated in the recesses of the gums and teeth. There, under favorable conditions, it multiplies and accumulates. The conditions for its establishment are apparently pyorrhoea alveolaris, gingivitis, caries of the teeth, and possibly other conditions where there is an accumulation of inflammatory exudate and débris. Both Hogue and Hinshaw failed to find it in normal mouths.

That is its most severe indictment. The general conception, however, seems to be that it is a scavenger, living on bacteria and possibly other foreign materials of the accumulations in which it is found and that it causes no harm to its host.

The fact of its presence in such a large percentage of such conditions, and only then, makes it advisable that it be held in mind until we know more about the genesis of pyorrhoea and kindred diseases of the gums.

TREATMENT

Prevention of the infestation would undoubtedly demand care to avoid taking into one's mouth any substance contaminated with the saliva of another and abstainence from mouth to mouth contact.

Eliminative treatment would involve the clearing up of the harbors of its life or the use of agents destructive to it.

Since both Hogue and Hinshaw found that a temperature of 45°C. was lethal to the organism it might be practical to apply such heat for the necessary ten minutes or so, as suggested by Rivas (1925) for treatment of intestinal protozoa. Hogue notes that prolonged administration of neosalvarsan had no effect upon it. The writer failed to eliminate it with emetine but saw a condition of acute gingivitis rapidly clear up and the trichomonads disappear during the course of thorough cleansing and washing of the gums and teeth with saturated solution of bicarbonate of soda.

It is probable that in deep pockets of pyorrhoea it would be very difficult to eliminate.

No conclusions may be drawn as to any measure of treatment at the present time, or whether it is worthy of any treatment. Further evidence and experimentation will be necessary.

TRICHOMONAS VAGINALIS

Although it is usually conceded that the vaginal trichomonad is distinct from those of the alimentary tract, it is necessary to consider *Trichomonas vaginalis* here because the question is not a settled one and it is not beyond the bounds of possibility that they may be the same, that what differences may be

made out are the result of environment and medium of growth. Should either or both of the other trichomonads be shown to be identical with it the species name will remain *Trichomonas vaginalis*.

First seen by Donne in 1837, this parasite is a very common inhabitant of the vagina, especially in the presence of an acid leucorrhoea, although this state may merely reveal it by calling for an examination. It is usually found in the vault of the vagina in an accumulation of greyish or white granular liquid material, in which desquamated epithelium and variable numbers of leucocytes are seen. Examined fresh in this material the organism is usually larger than either the intestinal or mouth trichomonad and it is not as actively motile in its natural state. It is seen crawling about in the débris and among the desquamated cells, frequently elongating and extending a "neck" which may be as long as the body is ordinarily. It twists and turns upon itself and seldom is of the active darting motion of *Trichomonas* in a liquid stool.

In material at the bottom of a culture tube it may have the same appearances, frequently being clumped in colonies, attached to solid materials. In liquid medium it develops a rapid swimming movement like the other trichomonads.

Its variation in size is from 5 by 10 microns to 15 by 25 microns or even larger, the largest sizes being in those preparing for or undergoing division.

It has four anterior flagella, undulating membrane,

axostyle, cytostome, nucleus, food bodies, etc., quite similar to *Trichomonas hominis* and *Trichomonas elongata (buccalis)*, for the details of which the reader is referred to Lynch (1922), Hegner (1925) or Wenyon (1926).

Wenyon (1926) finds the parasite very common in

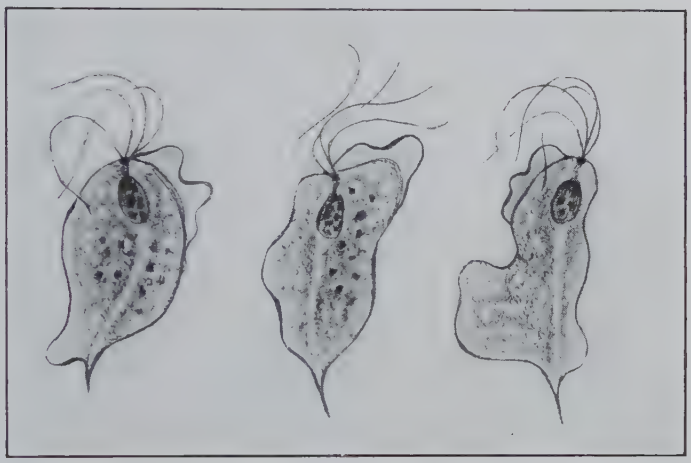


FIG. 25.—*Trichomonas vaginalis.*

Drawings of specimens stained by iron-haematoxylon. x 2000.

England and quotes Brumpt (1913) as obtaining 10% positive examinations in Paris. The writer has found it very common in women with leucorrhoea and in urine from such cases, the urine showing the organisms in centrifugalized sediment along with vaginal débris. It has not been seen in catheterized urine of women. Hogue (1927) found it only once in urine from 300 men and 333 women and then from

fecal contamination. Dock (1894) found the organism in clumps of pus cells and epithelium in freshly voided urine from a man on several occasions. The writer has seen it once, in catheterized specimen, in the urine of a man, out of several thousand examinations of the urine of men. Marchand (1894), Miura (1894), and Escomel (1927), have reported it in the urine of men. It has been cultivated by the writer, by Reuling (1921) (according to Hogue), by Hegner (1928), and others. The writer has secured prolific cultures in Hogue's ovo-mucoid medium. Hegner (1928), who has studied the spontaneous infection of the vagina of monkeys and has cultivated trichomonads from the monkey's intestine and vagina and produced experimental vaginal infection with them, makes no mention of any abnormal state associated with its presence. He finds the two morphologically similar and from this and the experimental vaginal infection with the intestinal trichomonad thinks they may be the same.

Davis and Colwell (1929) consider *Trichomonas vaginalis* a very common parasite in women with abnormal vaginal discharges and believe it to be an active factor in the production of vaginitis. They cultivated it best in 5% human serum in dextrose broth.

TRANSMISSION, HABITAT AND EFFECTS

The manner of transmission of *Trichomonas vaginalis* is unknown. Perhaps it may invade the

urethra of a man during coitus. The number of such occurrences is few enough to believe that such an accident may occur. Certainly, however, women do not contract it in a similar way.

If it is the same as *Trichomonas hominis* or *Trichomonas elongata (buccalis)* the method of infection of the vagina is obvious. This is so plausible that it furnishes one of the strongest reasons for suspecting this relationship.

The natural habitat is the vault of the vagina.

What harm it does there, if any, is unknown. As with other trichomonads the question is a debated one. The character of the material in which it frequently occurs is rather characteristic. It is not pus or mucopus, it is a greyish or whitish thick fluid composed of epithelium, variable leucocytes and flagellates. The state of the vagina may be that of a mild catarrhal vaginitis or more marked inflammation and there may be superficial excoriations of vaginal wall or surface of the cervix. The excretion or exudate may be irritating to the vulva. The common acid reaction of this material led the writer to make culture media slightly acid and to treat the infection with douches of saturated bicarbonate of soda solution. The organism has disappeared during such treatment. Study has not been sufficient, however, to warrant a conclusion from that.

Escomel (1917) recommends treatment with a 1 to 1000 solution of metallic iodine, freshly prepared, or with turpentine douches.

Mercurochrome has also been recommended. Davis and Colwell (1929) found it lethal to the parasite in 5% solution practically immediately. These authors also report that liniment of soft soap from 1% to 50% strengths kills the organism instantly, that 10% glycerine kills within one minute, that lactic acid stops motion in 2% solution within 45 seconds, that compound iodine solution has an instant killing effect in dilution of 1 to 100, mercuric chloride in 1 to 5000 solution and compound cresol solution up to 1 to 400 solution, having the same effect. It seems that there are a variety of therapeutic agents, which will destroy the organisms, available for vaginal application and that persistent treatment may be expected to eliminate them. The fact of elimination must be carefully determined.

CHAPTER XI

The Flagellates (Continued)

Chilomastix mesnili. Prevalence. Morphology. Cultivation. Transmission, Habitat and Effects. Treatment. *Giardia intestinalis.* Examination for Giardia. Morphology. Habitat and Effects. Treatment. *Tricercomonas intestinalis. Enteromonas hominis. Embadomonas intestinalis.*

CHILOMASTIX MESNILI

Chilomastix mesnili, although a uniformly common intestinal flagellate of man, was long confused with other organisms, particularly with *Trichomonas hominis,* and was accurately described by Wenyon only as recently as 1910. In the past few years it has become more familiar to the medical profession.

Reports from various regions give the incidence of this parasite generally the greatest of all the intestinal flagellates. Hegner (1925) found it in 7.7% in hospital patients in Central America, Hegner and Payne (1921) give it an incidence of 4% in 20,000 reported cases, Boeck and Stiles (1923) found 3.2% incidence in a survey of 8,029 persons. Jepps (1923)

found it in 3.6% of 674 Tamils and in 0.6% of 350 Chinese in Malaya. Barrow (1924) found the remarkable proportion of 535 instances of *Chilomastix* to 13 of *Trichomonas* and 10 of *Giardia* in 725 persons who harbored intestinal protozoa in 100%. Thomas and Baumgartner (1925) found 21% of the inmates of a New York state school for feeble-minded women to harbor this parasite. Smithies (1926) found it in only 2.6% of his 3780 patients with gastro-intestinal disturbance. Kofoid and Swezy (1920) note that it was found in 4.2% of returned overseas soldiers, in 3.5% of soldiers who had not gone out of this country, and in 5.3% of local residents. Hill and Hill (1927) found it in 24% of children of pre-school age in Porto Rico.

The writer (1928) found 7.5% infection, on the occasion of one immediate examination of purged stool of 1040 patients having some gastro-intestinal disturbance.

It is usually readily found, when present in active form, by the method given for the examination of stools. Its cysts may also be found in liquid or natural stool in variable numbers and at irregular intervals.

In active form, described as pearshaped, it is usually more the shape of a carrot. It is usually larger than *Trichomonas hominis* but averages about 10 to 15 microns in length, although forms much smaller and larger may be seen, especially in liquid stools or cultures. Its shape and form is constant as compared

to *Trichomonas hominis*, it being nonplastic, and unflexible. Its motion is always forward, the broader end being the head, and it distinctly rotates as it swims. This rotation is apparently due to the fact that the body is twisted or grooved in a spiral from before backward.

Arising from the blepharoplast, a granule or gran-

Fig. 26.—*Chilomastix mesnili.*

Drawings of trophozoite and cyst from specimens stained by iron-haematoxylon. x 2000.

ules just beneath the surface in the anterior extremity, are three flagella, which, in rythmic lashing, propell the body forward and brush food particles into the cytostome. These can be counted in sluggish or motionless specimens, by good lighting in ordinary

liquid preparation, or in stained preparations. Arising also from the blepharoplast is a flagellum which is directed backward in the cytostome and which waves or undulates in sinuous movement, synchronously with the three free flagella. The cytostome is a distinct groove from the anterior extremity over and into the surface of one side of the body for about one half of its length. This groove is supported by marginal fibers originating with the flagella.

The nucleus, not distinct, is rounded or ovoid, in the anterior end, just beneath the surface and back of the cytostome. In iron-haematoxylon preparations it has a definite rim, on which is irregularly distributed chromatin, and a central karyosome.

The cytoplasm is variously vacuolated and contains, usually, ingested bacteria.

The cysts were first described by Prowazek and Werner (1914), according to Wenyon (1926). The writer mistook them for cysts of *Trichomonas hominis* (1916). They are insignificant bodies, of "ground glass" appearance, apt to be taken for yeasts by the inexperienced. They are pear or lemon shaped, the anterior end being the narrower and often elevated by a "shoulder" above the the adjacent convexity. They are about 7 to 10 microns in length. In iodine preparations the nucleus and cytostome may be seen and sometimes they may be made out in the unstained specimen.

Proper staining by the iron-haematoxylon method is essential for studying the detail of these cysts.

Here the nucleus, the marginal fiber and flagellum of the cytostome, and the space between the anterior pole and the uplifted end of the cyst shell may be seen.

Chilomastix mesnili was first cultivated by Boeck (1921) in a medium composed of human blood serum one part, Locke's solution four parts, with 0.25 gram of dextrose to each 100 cc., incubated at 37 degrees C. in 5 cc. amounts of the medium in small test tubes. Boeck and Drbohlav (1925) and Thomson and Robertson (1925) report its cultivation on Boeck's L. E. S. medium, as described under *Endamoebea histolytica*. The writer has grown it in Hogue's ovomucoid medium, Boeck's L. E. S., and Tanabe's (1925) medium for trichomonads, composed of NaCl 0.7 gram, sodium citrate 1.09 grams, Loeffler's blood serum, dehydrated, 0.5 gram, white of egg 2 cc., distilled water 100 cc. In making this medium the salts are first dissolved, then the egg is added and shaken vigorously, finally the dehydrated blood serum is shaken with it.

Success with these and other mediums is not always the same. As with trichomonads, bacteria are uncontrollable and are unquestionably influential in the success or failure in carrying on cultures.

Division of the organism is by longitudinal binary fission, such as occurs in the case of the trichomonads. According to Kofoid and Swezy (1920) the nucleus divides by mitosis. Following nuclear division occurs the splitting of the cytoplasm, the

flagellar organelles being probably newly formed from the divided blepharoplasts.

TRANSMISSION, HABITAT AND EFFECTS

That *Chilomastix mesnili* becomes readily transmitted is shown by its widespread relatively high prevalence. Once looked upon as a tropical parasite, and possibly still to be regarded as of higher incidence in warm regions where sanitation is poor, it is able to survive more rigorous climates and to spread in disregard of modern sanitary facilities. The increase shown by Thomas and Baumgartner in institutional life indicates person to person passage, no doubt by unclean personal hygiene and the handling of food by carriers with unclean hands.

The fact that it has a cyst indicates this as the resistant and transmissible phase but it is not certain that the trophozoite may not pass to establishment in a new host, even through the stomach, in naked form. Experimentally it has stood exposure to hydrochloric acid even better than has *Trichomonas hominis*, which is believed to necessarily pass the stomach in active form. It withstood 0.1% and 0.2% solutions of the acid in 0.9% sodium chloride solution and was active after 1½ hours, but not in 0.3%. The active forms are almost immediately destroyed by water and they probably are not transmitted in that medium, although the cysts may be.

In addition to the transmission of the parasite from personal contact, the spread of the cysts from exposed excreta by flies no doubt takes place.

The writer (1928) found *Chilomastix mesnili* to be more related to city life, 80% of its hosts in his study being city dwellers as compared to 56% of *Trichomonas* carriers and to 70% of the flagellate-free. Males were a bit more frequently infected; in a clientele of about 60 females to 40 males, 51% of *Chilomastix* hosts were males. Perhaps men come more into contact with it by being in contact with more people.

As the age of individuals increases so does the frequency of the parasite, only 11% of *Chilomastix* carriers were below forty years of age and 55% below fifty, while of *Trichomonas* carriers 53% were below forty and 83% below sixty. This is consistent with the finding of Thomas and Baumgartner. The organism seems comparatively tenacious as a parasite. Although it varies in numbers from time to time it seems to maintain itself in the intestine for long or indefinite periods. One host is known to have harbored it for a period of fourteen years.

Of the *Chilomastix* carriers 40% showed a condition of gastric anacidity, while this was the case in but 19% of the flagellate-free. On the other hand, only 10% of them had an excess of gastric hydrochloric acid at the time of examination, in contrast to 25% of the free.

There was diarrhoea at the time of examination

or conspicuous in the history in 8% of the flagellate-free and 6% of those with *Chilomastix,* while constipation was the state of 74% of the parasitized, against 55% of the free.

Although recognized as an indefinite thing in itself, there was a clinical diagnosis of chronic cholecystitis in 37% of the carriers of *Chilomastix,* as compared to 22% of the non-parasitized.

There was undoubtedly a state, in patients of the later years of life, in which there was gastro-intestinal disturbance, but of no definite character, with achlorhydria and constipation as conspicuous features and in which chronic cholecystitis was commonly diagnosed, in which the presence of *Chilomastix mesnili* was an outstanding finding. In none, however, was the organism found in gastric, duodenal, or transduodenal biliary drainage.

Since this is the sort of clinical state in which the organism has been under suspicion by medical practitioners, these findings must be held in consideration for further information. The question is, which is the cause and which the effect. Is the prominence of the organism here because of the favorable conditions for it, later life, a lowered acid barrier in the stomach, a fertile field for its maintenance in a stagnant intestine content, or is the organism related directly or indirectly to the development of this abnormal state?

There were no other symptoms, signs or laboratory evidences of disturbances related in any way

to the presence of the organism, beyond the control group.

It should be recorded that none of these *Chilomastix* hosts suffered from arthritis deformans, since Barrow (1924) found 94% of 79 cases of this disease to harbor this parasite.

The natural habitat of *Chilomastix mesnili* is the colon, particularly the caecum. There it lives only as a lumen dweller, so far as is known. Wenyon (1926) found it in the lumen of the glands of the colon. It has not been observed as a tissue invader.

It is not known to cause any local degenerative or inflammatory change and the conjectured pathologic state produced by it is virtually narrowed down to a state of "toxemia," of which there is only circumstantial evidence.

Castex and Greenway (1925) are among the latest to promote such a rôle for the organism, they finding it in 118 of 614 cases of "chronic intestinal toxemia," in which they were of the opinion that the organism was the pathogenic factor and essential cause of the different toxic symptoms in the 49 cases of "pure infection."

TREATMENT

Preventive. Since *Chilomastix mesnili* thrives in spite of modern sanitary precautions against the spread of infection, it is to be suspected that person to person transmission is the important method in modern civilized communities. More careful observance

of rules of personal hygiene and, probably, the control of food handlers which harbor the parasite, seem, therefore, to be of particular importance in any expectancy of prevention of its spread. Proper sewage disposal and fly control are already well observed where it is prevalent, but no doubt are responsible factors in the spread of the parasite and for higher incidence in regions where these practices are not in force. Control of the spread of this parasite seems to be quite similar to that of *Endamoeba histolytica.*

Curative. There is nothing in the nature of a specific curative method of treatment or drug to be presented in this case.

The several remedies which are used against the intestinal trichomonad are used against *Chilomastix.* Among these the arsenicals have been used. In the test tube, however, neoarsphenamine in as strong solution as 0.45 gm. per 100 cc. of 0.9% sodium chloride solution did not kill this organism within an hour.

It should not be difficult to determine the effect to those arsenicals used in the treatment of syphilis, since a certain per cent. of the patients of any syphilis clinic harbor the parasite, and it is not difficult to determine its presence and continuance.

GIARDIA INTESTINALIS. (GIARDIA LAMBLIA. GIARDIA ENTERICA.)

Giardia intestinalis, formerly called *Lamblia intestinalis,* is the representative in man of a genus of protozoa having a number of species in animal

hosts. As pointed out by Dobell (1920) it was probably first seen by Leeuwenhoek, the father of Protozoology (1681), who found himself to be a host of it.

Lambl in 1859 and 1860, Grassi (1879), and Blanchard (1888) are the early students concerned with its history.

Until the past few years it was thought to be an uncommon parasite and there was little study of it except an occasional report of its presence in some person, usually a child, who had diarrhoea.

Of late, it has come into more general recognition as a very common intestinal flagellate, especially in children.

Kofoid, Kornhauser and Plate (1919), in one of the earliest studies of a large number of persons, found 1500 United States soldiers, 300 of whom had not seen foreign service, to harbor it in 6%. Hegner and Payne (1921) arrived at an average incidence in 20,000 reported cases of 12%. Boeck and Stiles (1923) give an incidence of 6.5% in 8029 persons, many of whom were children, examined by them. Thomas and Baumgartner (1925) found only 2.5% incidence in female institutional inmates. Maxcy (1921) found about 20% of 74 children between one and thirteen years of age infested. In England, Matthews and Smith (1921) found 14% of 548 children, Campbell (1921) found 16% of children under twelve years, and Nutt (1921) found 48.8% of institutional children and 23.9% of hospitalized children, to harbor the parasite.

In Porto-Rican children of pre-school age Hill and Hill (1927) found an incidence of 47.2%. The writer (1928) found an incidence of only 2.3% in a medical clientele of 1040 cases, in which, however, children were a small part of the number, and, even at that, 45% of the *Giardia* hosts were under twenty years of age.

It was long believed that *Giardia* infection of man was derived from other animals. At the present time there is no reason to suppose such to be the case, nor is there need to go outside humankind to find sufficient explanation of the source of the parasite. While many animals harbor giardias indistinguishable from that of man by the inexperienced, and some have species very closely similar at expert observation, it is generally considered by the best authorities that they are distinct species and that ordinarily the only one with which man is concerned is his own.

Institutional studies indicate that transmission is commonly by almost direct transfer from one person to another. Close personal contact with the infected leads to spread of the parasite regardless of modern sanitary precautions as to the disposal of sewage, fly control, etc. Undoubtedly, however, the contamination of food and drink by food handlers of unclean habits, the exposure of human excreta, without disinfection, to flies, and to drinking water, the use of human feces as fertilizer for garden stuffs, are all capable of and probably do play their part in transmitting the organism.

As with most of the intestinal protozoa the cyst is the transmissible form. The trophozoite does not appear in the natural stool and even when passed from the intestine, as in diarrhoea, it is so delicate that it does not live long and is apparently incapable of surviving exposure to the acid of the stomach for a sufficient period to allow it to pass alive to the duodenum.

It has been observed in stomach contents when there was no trace of free hydrochloric acid, but was almost immediately destroyed by gastric juice of normal acidity and by 0.1% hydrochloric acid in 0.9% sodium chloride solution. Although it is not impossible that some of the other encysting protozoa of man's intestine may pass in viable trophozoite form through the stomach at times, *Giardia intestinalis* apparently never does.

EXAMINATION FOR GIARDIA

The routine stool examination practice given under the amoebae is sufficient for determining the presence of *Giardia intestinalis* when it is appearing in stools. When there is diarrhoea it is usually present in active form, and a late specimen following a saline purgative will usually bring it out in this form. Otherwise it is only the cysts which appear and they irregularly or periodically, so that it is difficult to be certain that it is not present from even several examinations. In this case, except in liquid stool, it

is the cyst which must be depended upon for appearance.

In case of their presence, duodenal or trans-duodenal biliary drainage usually shows the trophozoites, not the cysts, in the duodenal content removed. The parasite became very prominent in the course of the recent commonly practiced trans-duodenal biliary drainage. The writer has seen it frequently in such material. Hollander (1923) found it in nine of one hundred and seventy such examinations. This is, however, not necessary as a measure for this purpose only.

The trophozoite has a form so characteristic that it should take only a picture of it to lead to identification on first observation. If a pear were split in halves longitudinally and inverted and the anterior half of the split surface scooped out to a shallow concavity, one would have the form of an inert organism. Assume that body as flexible and attach at the proper places four pairs of long flagella, which in movement cause the organism to vibrate rapidly as it swims and flexes its body, and one has the form and action of the organism.

The size of the active form is about 10 to 20 microns long by about half that width. Seen anteroposteriorly it is pearshaped. In lateral view the dorsal surface is convex forward and concave behind, the outline being a shallow S, while the ventral side shows the forward depression of the oral sucker, and a gradual tapering from its border to the tail, this in the

motionless organism. In that state the flagella may be seen to hang as long delicate fibrils, one pair from forward on the outer border of the sucker, two pairs from near the midline posterior to the sucker, and the fourth pair from the tail.

In motion the body vibrates rapidly with the action of the flagella and it frequently doubles upon

FIG. 27.—*Giardia intestinalis.*

Trophozoite and cyst as drawn from living unstained specimens. x 2000.

itself. After all other motion has ceased, the flagella which extend backwards along the ventral midline may continue to undulate.

In fresh unstained specimens the cysts are readily recognizable to one on the search for them, although they vary in number in the same individual and may be absent at times. There is some belief that they appear periodically as a habit of life of the organism.

They are about 8 to 15 microns long, are of an elongated ovoid shape with rather bluntly rounded ends, and are of a "ground-glass" appearance. In one end are the nuclei which may be seen on careful observation, reminding one of eyes. In iodine solution, showing the cyst wall, the brown body, with the nuclei and the flagellar apparatus more or less distinct, the cyst can hardly be confused with anything else. Uniformly sized ovoid "ground-glass" bodies with "eyes" in one end are likely to be *Giardia* cysts.

In preparations stained with the iron-haematoxylon method, specimens showing rather clearly the intricate flagellar system, the nuclei, the cytostome, the axostyles, are obtained without great difficulty.

The oral sucker is composed of two discs or shields, outlined by stiff marginal fibers. There are two nuclei in the cytoplasm, one about the middle of each shield, each with central karyosome. The anterior pair of flagella arise from the blepharoplasts and after passing forward for a way, decussate and round the margins of the oral sucker, becoming free after traversing about one third of the margin. The two middle pairs of flagella come from about the junction of the two lateral shields posteriorly, one pair passing directly backward, the other diverging. The two posterior flagella are the free fibrils, coming from that extremity of two stiff fibers, the axostyles, passing straight up the middle of the body to the blepharo-

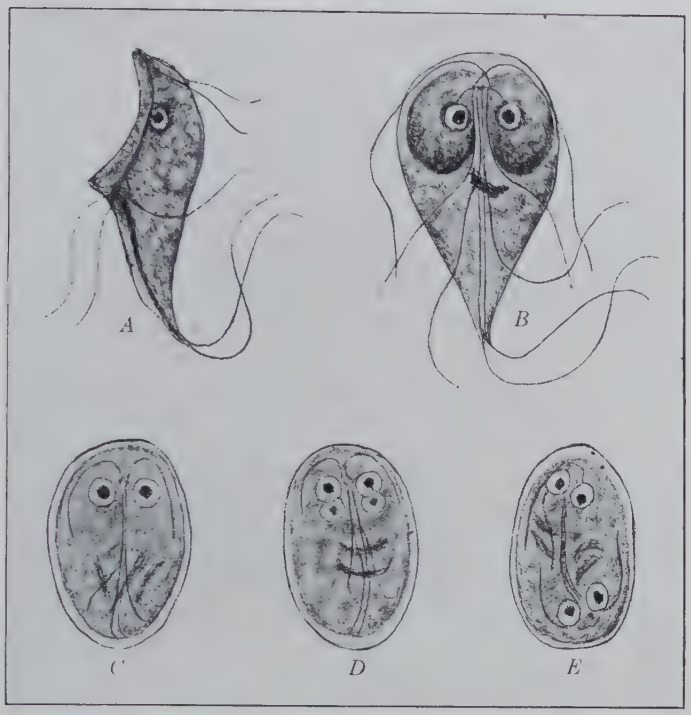


FIG. 28.—*Giardia intestinalis.*

A and *B*, trophozoites as seen from lateral and anterior view. *C*, *D* and *E*, cysts in different stages of development. Drawings of specimens stained by iron-haematoxylon. x 2000.

plasts. A "parabasal" body occupies an oblique position in the cytoplasm posterior to the junction of the shields. In the cyst the stage of development determines the number and position of the nuclei and the neuro-motor apparatus. The oral sucker is not seen although some of the marginal fibers of the two shields may show. The nuclei may be two,

in one end of the cyst, in the single organism, but commonly are four in number, usually all grouped but at times two in one end and two in or toward the other. The fibrils of the flagellar apparatus are twisted and wavy lines, to be studied by the very careful investigator. The cyst is, therefore, a multiplication cyst as well as a resistant phase. It is readily stainable and its structure recognizable. For more minute structural detail the reader is referred to works, such as by Wenyon (1926), dealing more at length and minutely with such matters than is here desirable.

The organism divides within the cyst, by binary fission, after mitosis of the nuclei, and two offspring are liberated at excystment, on reaching its natural habitat. Similar longitudinal division has been described in the active form and this probably occurs within the intestine, since excystment in the same individual does not seem likely. Under conditions of observation of the parasite from duodenal contents and liquid stools division is not apt to be seen. Cultivation of the organism has not been done and its reproduction has not been extensively followed.

The cytoplasm is of alveolated, finely granular character, the dorsal part more dense than the ventral. It is not vacuolated or filled with bacteria as in the other flagellates. Evidently its food is taken in soluble form and the materials have not yet been supplied properly for the growth in culture.

HABITAT AND EFFECTS

The home of *Giardia intestinalis* is the upper small intestine, apparently mainly the duodenum, instead of the caecum as with *Trichomonas* and *Chilomastix*. Here the organism finds the environment and materials suited to its well being and multiplication. That its needs are radically different from those of other flagellates is given evidence by its structure, the absence of bacteria from its body, and its failure to survive in media which will grow others well. As it passes down the intestine it becomes encysted and prepares for transference to a new host in mutiplied form.

It is believed that the oral sucker is the means of maintaining its position and it has been seen attached by this apparatus to the epithelial surface of the mucosa. It has not been ascertained that it damages these cells or excites any reaction on the part of the mucosa, although some believe that it irritates the epithelium and stimulates mucus production.

The term "giardiasis" has, at the present time, no definite meaning beyond the presence of the parasite in the intestine. In children *Giardia* has been suspected of an etiologic rôle in persistent diarrhoea, with clear or yellowish mucus, in which are seen large numbers of the active form of the organism, conspicuous in the stool.

Hill and Hill (1927) in a survey of children of pre-

school age found that of the 47.2% infected with *Giardia intestinalis,* 57.6% had chronic or recurring diarrhoea, as compared to a similar history in only 24.2% of the flagellate-free, and in 34.8% and 27.9% of the carriers of *Chilomastix mesnili* and *Trichomonas hominis,* respectively.

Zahorsky (1928) is convinced of the production of intestinal disturbance in children.

The very common occurrence in children, especially under crowded conditions, without any evidence of the production of any disturbance, brings serious question as to whether it ever causes trouble. It is most common between the ages of one and ten years and this is the period when intestinal upsets are most apt to occur. Merely its presence in a case of diarrhoea or dysentery does not prove it to be a pathogenic factor.

There is, however, a very strong belief in the medical profession that giardiasis signifies a state of upper small intestine irritation with "indigestion" and chronic or recurring diarrhoea, with mucous, but never bloody, stool, occurring particularly in young children. Such studies as that of Hill and Hill (1927) showing diarrhoea conspicuous, much beyond the controls, is strong evidence in support of this belief. Similar repeated studies with like results would go far toward establishing the pathogenicity of the organism in a definite clinical syndrome. From a working viewpoint it is, perhaps, not unwise or unreasonable to assume that such a condition occurs,

unless it is more clearly shown that such is not the case.

As to the infestation of the biliary tract and gallbladder there is further suspicion, and need of further investigation. The presence of the organism in the bile and duodenal material from a trans-duodenal biliary drainage does not prove that it came from the biliary system. However, it has been observed, according to report, within the gallbladder, removed by operation, on several different occasions, and it is believed by some that it is related to a state of chronic biliary tract inflammation with jaundice, this state associated with later life and a sluggish or costive bowel.

In the writer's experience it occurs in the young and the old, 27% of the *Giardia* hosts, all being children, having diarrhoea, as compared to 8% of the flagellate-free, 6% of the carriers of *Trichomonas hominis* and 7% of carriers of *Chilomastix mesnili,* while 40%, these all being adults, were constipated.

TREATMENT

In a general way the prevention of this infection should follow the same rules as for *Endamoeba histolytica* or *Chilomastix mesnili.* Although, no doubt, commonly transferred in the encysted stage by exposure of unsterilized human excreta to drinking water and to flies, and the use of it in garden stuff fertilization, in modern community life and in in-

stitutions where these conditions are controlled it still thrives and is readily transferred by direct contact, unclean personal habits, direct finger to mouth transmission, and the contamination of food by its handlers.

The ways and means of prevention of its spread are obvious, although no doubt difficult of enforcement under crowded conditions, particularly in a certain type of institution for boarding homeless children.

Although generally believed, it is not definitely shown that some of our domesticated animals and pets may not carry giardias transmissible to man. If it should be the case that some infections may be of this source, the "dirty" age of childhood, particularly, would need to be protected against this possibility.

Specific eliminative treatment has proven largely unsatisfactory to the present.

It should be borne in mind that its absence from the intestine is not provable by single or even a repeated examination, and that a supposed elimination may not be real. Prolonged observation is necessary.

Various drugs have been reported as efficacious by some and as of no effect by others. Among these are quinine, emetine, bismuth subnitrate, beta-naphthol, methylene blue, and the salvarsans.

Zahorsky and McLoon (1927) recommend bismuth salicylate, given in 5 grain (0.3 gram) doses three times a day to a child of six years.

Hollander (1923) after failing to effect the presence of the organism with arsphenamine injected in the usual way at 3 to 5 day intervals, alternated these injections with duodenal lavage with magnesium sulphate solution in one case, the patient remaining free of the parasite to examination of duodenal contents over a period of six months.

Kofoid (1919) et al., freed rats of their natural infection by giving arsenobenzol in one intravenous injection of four to eight times the ordinary human dose, pro-rated by weight, the rat successfully standing the treatment.

At the present time eliminative therapeutics is in an uncertain and experimental stage.

On this account and because of the uncertainty as to the necessity for treating it at all, ill advised therapeusis should be avoided and any measures used in the cases of young children should be very thoughtfully regarded first.

TRICERCOMONAS INTESTINALIS

In addition to the more common and larger intestinal flagellates, man harbors several less common and smaller forms.

Among these is *Tricercomonas intestinalis* (Wenyon and O'Connor, 1917), found in widely separated regions but apparently of comparatively rare occurrence. It is subject to the likelihood of confusion with other small flagellates but the description of

Wenyon and O'Connor has been confirmed by the writer (1922). It has also been observed in this country by Boeck (1924).

The organism is from 4 to 7 or 8 microns in longest diameter, it being almost rounded or slightly ovoid. It is very active in fresh preparation, swimming jerkily and rotating at the same time. The cytoplasm is alveolated and contains bacteria. It anchors itself by a caudal process, as is the habit of flagellates when feeding.

The anteriorly directed flagella move rapidly and are not to be counted with certainty in an active stage. At times they can be seen to come from a minute knob, the blepharoplast. There is a recurrent flagellum which arises at the same place passing posteriorly over the surface of the body, to which it is adherent for about three fourths of the body length, terminating in a free extremity from the posterior quarter. In motion there is a slight protoplasmic projection where this flagellum leaves the body. The nucleus is not visible in the unstained. The cysts are minute bodies, slightly smaller than the trophozoite, and possess a nucleus in each end, as seen in stained specimens. They are ovoid in shape and have a definite cyst wall.

In preparations stained by Wright's blood film stain the flagella could be observed and counted. In iron-haematoxylon preparations the nucleus was seen at the base of the flagella. It was ovoid and had a central karyosome and definite rim.

The organism grew well for four days in ascitic fluid diluted with 4 parts of 0.9% sodium chloride solution, ranging in size from 3 microns to 10 microns in the dividing forms. Division was by longitudinal fission, the nucleus apparently passing

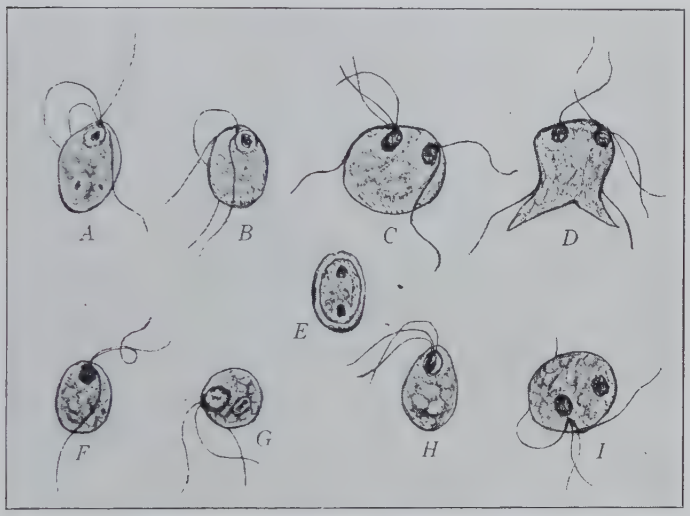


FIG. 29.—*Tricercomonas* AND *Enteromonas*.

A, B, C and *D,* trophozoites, *E,* cyst, of *Tricercomonas intestinalis*. *F, G, H* and *I, Enteromonas* from the guinea pig. *C, D* and *I,* dividing forms. Drawings from specimens stained by iron-haematoxylon. x 1600.

through mitosis, the two halves of the dividing body being equipped with nucleus and flagella before the body completed division.

The parasite has been cultivated in Boeck's L. E. A. medium by Boeck (1924) and Thomson and Robertson (1925).

The writer did not find it in duodenal drainage of the host and assumes it to be a large intestine inhabitant.

Nothing further is known at the present in regard to its life history or relation to its host.

ENTEROMONAS HOMINIS

Subject to confusion with *Tricercomonas intestinalis* and with *Embadomonas intestinalis* as a small flagellate, named *Enteromonas hominis* by Da Fonseca (1915).

The size, motion, shape and nucleus are quite similar to *Tricercomonas intestinalis* but there appear to be only two anteriorly directed flagella and the recurrent one does not appear to be definitely fixed into the body.

Similar organisms have been seen by several observers in widely separated regions. The writer (1922) observed what was apparently a species in the guinea pig and named it *Enteromonas caviae.*

EMBADOMONAS INTESTINALIS

Of better standing and wide distribution, though not as common as the larger flagellates, is another minute organism, *Embadomonas intestinalis,* found by Wenyon and O'Connor (1917) in Egypt. It has been seen and described by Kofoid, Kornhauser and Plate (1919) and by Hogue (1921) in this country, the latter investigator cultivating it in several media.

The organism measures about 5 to 7 microns in length, is pearshaped, has a conspicuous cytostome and two flagella, the anterior of which is directed forward and used in locomotion, the other directed back through the cytostome and apparently used in feeding. It moves in a jerky manner.

The cytoplasm is alveolated and vacuolated and

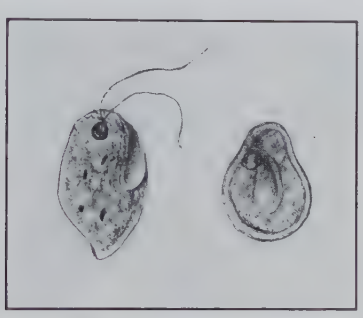


FIG. 30.—*Embadomonas intestinalis.*

Drawings of trophozoite and cyst from specimens stained by iron-haematoxylon. x 3800.

contains bacteria. The nucleus, seen only in iron-haematoxylon stained specimens, is at the base of the flagella, is rounded, with definite membrane and central karyosome.

The cysts are slightly smaller than the trophozoite, are pearshaped refractive bodies, resembling the cysts of *Chilomastix mesnili,* but smaller.

Although attention has been called to the organism usually in conditions of diarrhoea, there is no definite information as to its exact habitat in the intestine or any local or systemic effect of it.

CHAPTER XII

The Ciliates

Classification. General Characteristics. *Balantidium coli.* Prevalence. Transmission. Morphology. Habitatat and Effects. Balantidial Dysentery. Chronic Balantidiosis. Treatment. *Balantidium minutum. Nyctotherus baba.*

CLASS: CILIATA.
ORDER: HETEROTRICHIDA.
GENUS: BALANTIDIUM.
SPECIES: *B. coli.*
B. minutum.
GENUS: NYCTOTHERUS.
SPECIES: *N. baba.*

THE CILIATES (CILIATA)

Of the class of Protozoa known as the *Ciliata,* commonly called the *Infusoria,* there are two genera with representatives in man's alimentary tract: *Balantidium,* represented by *B. coli* and *B. minutum,* and *Nyctotherus,* represented by *N. baba.* There are other species described but not well accepted.

The *Ciliata* are characterized by rather large size, complete surface covering of short filamentous or

hairlike processes, called cilia, a prominent mouth, contractile vacuoles, and two nuclei, the macronucleus or meganucleus, the larger, having to do with the somatic life of the organism, the micronucleus concerned in germination.

The cilia are set in the ectoplasm of the body, through the surface, in rows. Their movement is rythmical and reminds one of a grain field with wind waves. Except those of the cytostome area they are purely for the purpose of locomotion, serving to propel the organism rapidly forward and at the same time rotating it slowly, by means of the creation of currents.

The cilia in the depression around the cytostome are longer and serve for food gathering and introduction into the mouth.

The mouth or cytostome is conspicuous as a funnel-like indentation in one surface, the ventral, and from it food particles are carried into the endoplasm through a short oesophagus.

The ectoplasm is more dense than the endoplasm, which is vacuolated, the vacuoles serving as stomachs for the digestion of bacteria and other food materials.

The contractile vacuoles, seen only in this class of alimentary tract protozoa, are present in many free-living forms of protozoa. They pulsate rythmically and are related to the circulation or metabolism of the organism.

At the posterior end of the body is a small indenta-

tion, the excretory pore, through which the waste products are cast out.

The macronucleus, the large somatic nucleus, lies deep in the central part of the body, is bean shaped, and close to it, in the depression, lies the small micronucleus.

The whole body of the organism is somewhat pear-shaped, the smaller end being anterior. It is of fairly constant shape but is somewhat plastic and may show some degree of flexion and elongation in forcing its way through compact solid materials.

Those who have little or no opportunity to study these parasitic organisms, will be able to familiarize themselves with the general form and thereby avoid confusion with the flagellates by studying the common free living kin, *Paramecium,* in pond water or grass infusion.

BALANTIDIUM COLI

The most common ciliate of man's intestine, and the most conspicuous in size, is *Balantidium coli,* discovered by Malmsten in 1856 in two patients with dysentery in Stockholm. This is the only one familiar, even by name, to any considerable number of physicians or medical laboratorians.

At that it is not a common parasite. While it has been reported of world wide distribution, and is, by Walker's (1913) report, relatively common in the Philippine Islands, it is a rare parasite of man in this country. Craig notes that it has been seen in man

in Arkansas, California, Iowa, Louisiana, Minnesota, North Carolina, New York, and Oklahoma, and that, although he has done many thousand examinations of stools, he has encountered the organisms only a half dozen times and never in any individual examined in the United States. Deeks (1925) notes its presence in hospital laboratory reports in central America, in 13 cases in 1923, 88 cases in 1924, and 128 cases for 1925, while Aguilar (1925) reports that during the period from August 30th to October 2nd, inclusive, 40 cases of balantidic infection were admitted to the Quirigua Hospital, Gautemala, this being 10% of the admissions. All of these patients were apparently admitted on account of some other ailment, all gave a questionable history of diarrhoea, and fourteen of them showed pus or pus and blood in the stool. He believes that balantidial infection is most prevalent in this region following the rainy season and that its source is the common domestic pig. In the Annual Report of the United Fruit Co. for 1928, J. C. McDaniel, reports finding *B. coli* in 0.41% of 5587 stool examinations. The only instance of its occurrence in man in this country observed by the writer was in an Arabian immigrant.

Balantidium coli is a fairly common intestinal parasite of the domestic pig and it is believed by many that this animal is the natural host, that man is a host by accidental transmission from the pig. It is highly probable that the isolated cases of infection of man are from this source and entirely likely

that the pig is the main source of spread. It is also a parasite of the intestine of monkeys.

It is transferred in the encysted phase from the feces of swine, probably of man, and possibly of monkeys, to man by ingestion of contaminated substances or, perhaps, directly from hand to mouth.

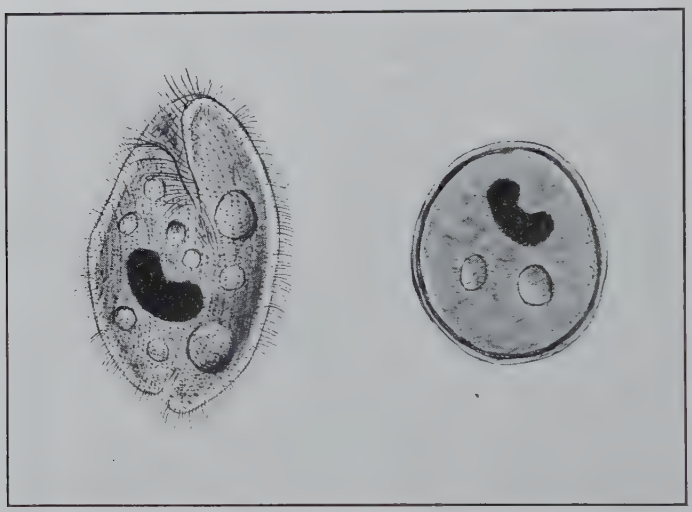


FIG. 31.—*Balantidium coli.*

Drawings of trophozoite and cyst from specimens stained by iron-haematoxylon. x 550.

Balantidium coli is a large organism, by comparison to other alimentary tract protozoa, the original description of Malmsten giving it a size of 60 to 100 microns long by 50 to 70 microns wide, small and large forms occurring in individual infections, according to Craig (1926).

It is roughly pearshaped, the narrower end being

the head. The surface is entirely covered by diagonally longitudinal rows of fine cilia arising in grooves in the ectoplasm. To one side of the anterior end is a funnel-like depression, the peristome, leading into the cytostome or mouth. The peristome is lined by longer and more delicate cilia. These gather food and by selection, or "taste" as Dobell (1921) expresses it, pass on the desirable to be ingested through the cytostome and short tubal gullet into the endoplasm, where it is digested in the vacuole stomachs. The food consists of a variety of substances from the intestinal contents and tissues of its habitat, the recognizable bodies being bacteria, starch granules, red blood corpuscles, leucocytes and tissue fragments.

The cytoplasm is mainly granular, alveolated and vacuolated endoplasm, with a thin clear zone of ectoplasm beneath a membranous pellicle.

The contractile vacuoles are two in number, one in the dorsal region, slightly anterior, the other near the posterior end. They pulsate slowly and rythmically and are not easily made out at all times. The excretory pore is a small oblique aperture in the terminal end.

The macronucleus, a large body of kidney-like or sausage-like shape, lies near the middle of the body and in its bend is the minute micronucleus. In stained specimens the chromatin of the macronucleus is a mass of compact granules. The micronucleus may be so closely in contact with the larger nucleus as to be obscured.

The motility of the organism is free swimming forward with slow rotation, the locomotive cilia waving rapidly and rythmically, the organism remaining of constant shape, although it is plastic and may flex and elongate in pushing its way through compact débris.

Multiplication is by transverse binary fission, the micronucleus dividing by mitosis, the meganucleus amitotically (Dobell, 1921), the cytoplasm constricting transversely and forming two offspring, the posterior one necessarily forming new anterior parts and both undergoing more or less reorganization. It has been cultivated by Barret and Yarborough (1921) in a medium composed of 1 part of inactivated liquid human blood serum to 16 parts of 0.5% sodium chloride solution, incubated at 37°C.

The cyst, formed as the organism passes down the colon, is more rounded or ovoid, and has a definite wall. The body loses its food bodies and vacuoles and becomes compact and granular. It shows more or less cilia and other characteristic features in the newly formed cyst, in fact it still revolves for a short time within the thickwalled space. Older cysts may show only the shell, the body, and macronucleus of the organism. The cyst measures 45 to 65 microns. It survives exposure for some time outside the body in a moist state.

The active form may usually be seen at any time in feces of the infected, varying in number and ac-

tivity, being more conspicuous in diarrhoea or dysentery. The cyst is not always present, even in the natural stool.

Both are subject to ready observation and identification in fresh unstained preparation, the structural detail being subject to complete study only in well stained specimens, such as from the iron-haematoxylon staining.

The inexperienced should have no difficulty in recognizing it in stool examination; a knowledge of the existence of such an organism, a picture of it, and observation of it in fresh stool, should be all that is necessary. One should beware, however, of the common occurrence of similar free living forms which may occur in stale stools or in stagnant solutions.

HABITAT AND EFFECTS

Balantidium coli occurs as a parasite of the colon of man, after it has hatched from ingested cysts. Its activity is mainly in the caecum and it naturally encysts as it passes down the intestine. It may be seen active in any part, however, of the colon.

The behavior of the organism and its effects upon the host are not unlike the case of *Endamoeba histolytica*. It is a parasite therefore of considerable interest in medicine, and balantidiosis of the intestine of man has a significance akin to intestinal amoebiasis. In fact the different phases of intestinal amoebiasis have their counterparts in intestinal bal-

antidiosis; there are the two states, chronic balantidiosis and acute balantidial dysentery.

BALANTIDIAL DYSENTERY

The acute phase of the disease is the unusual consequence of infection, the acute attack, or the end result.

In this condition the organism is known to be a tissue invader. Manlove (1917) records that balantidiosis is a relatively unimportant cause of death in the Philippines, but may cause considerable morbidity, being characterized by colitis varying from simple catarrh to deep ulceration. Strong (1904) reviewed 127 cases in the literature of that time, with 35 deaths and 32 autopsies, including one of his own, of which 28 showed ulcerative colitis and 3 chronic catarrhal colitis. Walker (1913) produced experimental infection of monkeys with the organism from man and pigs and studied the evolution of the experimental disease.

He found that *Balantidium coli* does not enter through the lesions of colitis of other causes but through the sound intestinal epithelium, becoming the primary etiologic factor in balantidial dysentery. The early lesions consisted of hyperemia with or without punctiform hemorrhages, showing histologically, vascular dilation, minute hemorrhages, round-cell infiltration and eosinophilia. Some of the later submucosal abscesses were bacteriologically

sterile. The epithelium in early infection was intact except for mechanical injury, attributed to the entrance of balantidia or to minute hemorrhages, there being no exudation or ulceration.

Strong found the organisms invading the mucosa,

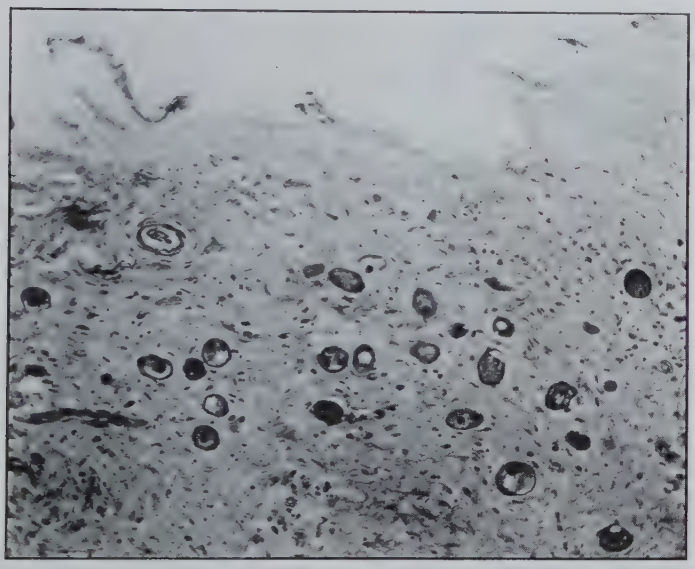


FIG. 32.—BALANTIDIAL COLITIS.

Group of *Balantidium coli* in the tissues of the submucosa below ulcer of the mucosa of the colon. Photomicrograph x 43.

submucosa and muscular layers. They were less numerous in the necrotic areas and were found in the margins of healthy tissue around these areas. The capillaries of the mucosa and submucosa were dilated, contained balantidia, and often showed hemorrhage. The amount of infiltration was small about

single parasites in the tissues, but was well marked around collections of the organisms. The ulcerations were undermined and often extended to the

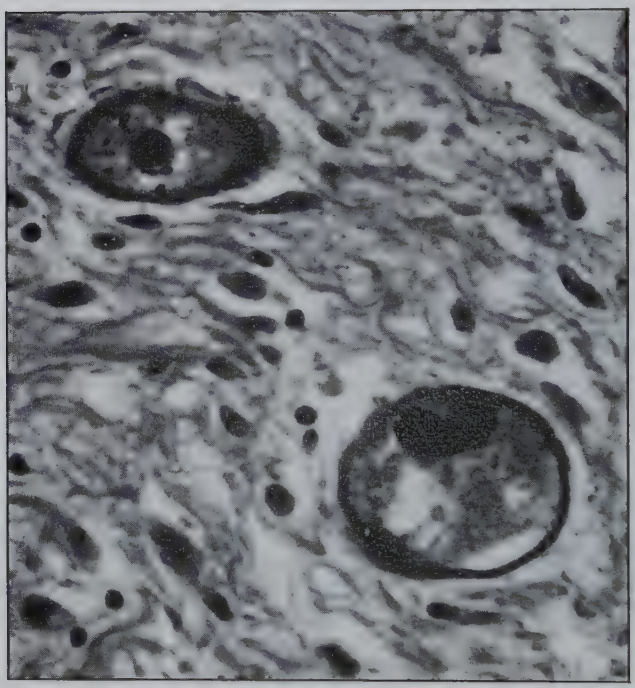


FIG. 33.

Balantidium coli within the tissues of the submucosa of the ulcerated colon in balantidial dysentery. Photomicrograph x 475.

muscular coat. The lymph nodes were hyperemic and enlarged. No parasites were found in any tissues outside the large intestine.

From these and other investigators it may be determined that *Balantidium coli* is capable of primary

penetration of a normal epithelial surface of the colon and of traveling in the tissues below. It excites a reaction of an inflammatory nature, including hyperemia and mononucleosis, although some record eosinophilia. Hemorrhage and necrosis occur, with the production of necrotic abscesses of the submucosa, which open through the mucosa to the surface, producing an undermined or flask shaped ulcer. The organisms may be seen singly, wandering afield from these primary foci, exciting little apparent reaction except as they accumulate. They occur in tissue spaces, the lymphoid follicles, lymph and blood vessels. As they multiply the abscesses and ulcers are extended and may become large and deep, even into the muscle coats, remaining, like amoebic ulcers, undermined, ragged, bowl-shaped. The manner of tissue destruction is not proven. Glaessner (1908) extracted a haemolysin but no proteolytic ferment. It is not unreasonable to assume the secretion of a histolyzing ferment, in comparison to *Endamoeba histolytica* as the direct agent of tissue destruction.

This tissue invasion, destruction, and production of characteristic ulcers, may occur from the caecum to the rectum, the larger and older lesions most common in the former part. When active and extensive particularly, probably when the rectum is involved, we have the clinical state of balantidial dysentery.

Here lasting from several days to several weeks there may be numerous stools, of a bloody liquid

character, or of blood-stained mucinous material, or liquid feces without gross blood. The individual may have more or less tenesmus, griping, abdominal discomfort, pain, and tenderness. Depending on the

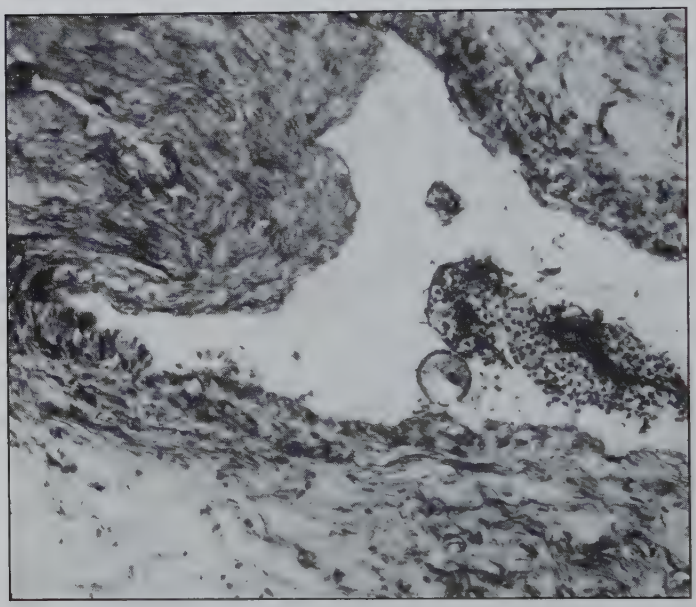


FIG. 34.

Balantidium coli within a vein, containing a thrombus, in the subperitoneal coat of the colon in balantidial dysentery. Photomicrograph x 96.

activity and length of the course, there may be irregular fever, weakness, loss of weight, various organic disfunctions, exhaustion, and even death.

The diagnosis depends upon the identification of the parasite in the stool, it always being present in

active swarms in the stool when diarrhoea is produced by it. The mucinous material shows the greatest numbers.

Attacks of balantidial dysentery may spontaneously terminate in apparent recovery and after a period, in which constipation is likely, an acute attack may recur. Whether the organisms remain within the colonic wall in these normal intermissions or in case of apparent recovery or whether they are then only lumen dwellers is unknown. We do know that ulceration may be present without diarrhoea, however, just as in the case of intestinal amoebiasis.

CHRONIC BALANTIDIOSIS

Much more common than the state of balantidial dysentery is that in which there is an infection with *Balantidium coli* without dysentery or even any apparent disturbance due to it. It is unknown whether in the "carrier" state without symptoms of disease the organism is purely a lumen dweller. In the comparable state in the pig, which is the rule, that is apparently the case, although ulceration of the colon of the pig may be produced, according to Brumpt (1909). Haughwout (1918) reports *Balantidium coli* in sections of pig's intestine in the tissues and blood vessels, and gives the microscopic picture of the infection as similar to that seen in human balantidiosis. We may, it seems, infer that the organ-

ism may dwell in the colon of man as a harmless parasite.

Walker says that the latency prevalent in balantidiosis of man is due chiefly to the fact that the patient, although parasitized, is not "infected," but in part to the chronicity of the ulcerative process in infected cases. He further states that a person infected with *Balantidium coli* is liable sooner or later to develop balantidial dysentery.

The state of infection in which there is a chronic or mild and continued process of invasion and ulceration is naturally less known than the comparable case of chronic intestinal amoebiasis, although when symptoms of progressive disease are present they may be more pronounced. Not uncommonly there is a chronic and intractable diarrhoea, and emaciation and anaemia may be conspicuous in the long standing case. Individuals showing symptoms of the infection commonly run an intractable course of chronic illness in which periods of dysentery alternate with those of comparative well being or constipation.

TREATMENT

Preventive: Since it has been shown that the infection of man may come from the pig, and that swine are commonly carriers of *Balantidium coli,* it necessarily follows that those who handle these animals or are in close contact with them must exercise care to avoid contamination of any object which is placed

in the mouth by feces of this animal, if this danger of contracting the infection is to be avoided. A similar case may possibly be that of the monkey, since this animal has been shown to harbor the infection naturally.

Prevention of the spread of infection among human beings is, fortunately, not a matter for important attention in this country or in most modern civilized countries. Where the infection is prevalent, however, this is one more incentive for the application of recognized methods of prevention of the swallowing of human excrement.

Curative: Specific eliminative treatment is in somewhat of a state of uncertainty. The older remedies used are quinine, 1 to 1000, iodine, 1 to 10,000, silver nitrate, 1 to 3000, tannin, etc., used by rectal injection. Mason (1919) injected rectally oil of chenopodium, 60 minims in one-half once of olive oil, with apparent success in one case. Emetine and other preparations of ipecac are largely discredited, although some have thought they had a good effect. Dobell (1921) thinks that the best chances of success lie in the use by rectum of concentrated solutions of quinine, such as used by Lanzenberg (1918), quinine hydrochloride 0.75 gm. in 300 cc. of water, since "rectal injections of quinine often appear to relieve the symptoms of balantidial dysentery, and to reduce the number of parasites, even when they do not effect a radical cure."

Haughwout, Domingo and deLeon (1920) reported

an apparently successful elimination of *Balantidium coli* from a case of balantidial dysentery in extremis by the use of benzyl benzoate. The drug was started in doses of 10 drops of 20% alcoholic solution, given in water three times a day. The same afternoon the patient seemed to have experienced some relief and he was distinctly better the next day. The following day the dose was increased to 15 drops. Two days later the bowel movement was more feculent and natural. He continued to improve and four days after the first increase the dose was raised to 20 drops. Balantidia appeared in the stool the last time sixteen days after beginning the treatment, at which time the patient was apparently recovered. The drug was continued altogether twenty-two days, when the occurrence of vomiting and pain caused it to be stopped. The patient died eleven days later of an unrelated disease. No balantidia were found in the content of the bowel, or in feces the day before death, and extensive section of the scarred colon failed to show any parasites in the tissue.

Aguilar (1925) reports that in his experience in Guatemala, stovarsal apparently had a specific action in the destruction of the organism in the intestine. He recommends the reduction of carbohydrate in the diet and the replacement of it largely by green vegetables and fresh fruits. Stovarsal is given in a 0.25 gram tablet, two or three times daily, after meals, for a period of seven days. After five days treatment

in forty cases the stools of all were negative for balantidia. Some of these cases examined two months subsequently still showed none of the parasites.

Greene and Scully (1913) recommend the following treatment, to be continued until the patient is free of the parasite. Two and one-half quarts of whole milk per day, given in small portions at regular intervals, supplemented after several days by one or two soft boiled eggs. Bismuth subnitrate in 15 grain doses for the first few days, to relieve cramps and diarrhoea. When the bowels become sluggish stewed fruits are given.

It is to be hoped that some one or more of these measures may stand the test of time.

BALANTIDIUM MINUTUM

Balantidium minutum was discovered by Schaudinn in 1899. He found it in two patients in Berlin, both suffering from diarrhoea. Whether it is a pathogenic species is unknown.

The body of the organism is more nearly of a pear-shape than is *Balantidium coli,* and it is smaller, measuring 20 to 30 microns by 14 to 20. The anterior end is more pointed and the mouthpiece is longer and relatively more prominent. The peristomal groove extends from the anterior extremity deeply into the body, to about the middle, and is lined by long delicate cilia. The surface cilia are longer

and more delicate than on *Balantidium coli.* There is only one contractile vacuole, it being situated on one side of the rounded posterior end of the body.

The macronucleus is rounded instead of kidney shaped, measures 6 to 7 microns in diameter, has a definite rim and its chromatin is more loosely arranged in granules through the nuclear network. The micronucleus is about 1 micron in diameter, spherical, and lies in front of the macronucleus. The cyst is usually ovoid. Multiplication is by transverse fission.

Our knowledge of this rare small ciliate is meagre and it is of only scientific interest, if in reality it is a parasitic species.

NYCTOTHERUS BABA

As with *Balantidium minutum, Nyctotherus baba* is more of academic than practical interest, it having been described in only one case, and that by Schaudinn also, in 1899. This patient suffered from diarrhoea and it was one of the two that harbored *Balantidium minutum.*

This organism is beanshaped, measuring 26 to 28 by 16 to 18 microns, being even smaller than *Balantidium minutum.* The mouthpiece extends from the anterior end backwards into the body where it enters the endoplasm through a short, oblique, narrow gullet. The peristome is lined by long delicate cilia, the body covered by short fine processes. As in

Balantidium minutum there is only one contractile vacuole, at the posterior end. The meganucleus is spherical, 6 to 7 microns in diameter, is about the middle of the body, and the chromatin is arranged in several large lobes. The micronucleus is about 1 micron in diameter. Oval cysts were seen by Schaudinn, possessing the characteristic nucleus.

CHAPTER XIII

The Coccidia

Classification. General Considerations. Life History. Habitat and Effects. *Isospora belli. Isospora hominis. Eimeria gubleri.*

CLASS: SPOROZOA.
ORDER: COCCIDIIDA.
GENUS: ISOSPORA.
SPECIES: *I. belli.*
GENUS: EIMERIA.
SPECIES: *E. gubleri.*

THE COCCIDIA (COCCIDIIDA)

The coccidia are very close akin in the protozoal world to the parasites of malaria and have a somewhat similar life history, although it is carried out within one host instead of requiring two for its completion, as in the case of the malarial parasite. They are, consequently, obligatory parasites, spending a certain part of their life cycle within certain cells of their host's body, and are, therefore, always and essentially pathogenic organisms.

While the class is widespread in the animal king-

dom and of considerable commonness in some host species, coccidia of man are not common. In fact, while not rarely found in certain places, in this country and in most they are rare parasites.

The complete life history of none of the species of man has been observed and it is necessary, in order for an understanding of its probable nature, to give the cycle of a typical species, such as has been completely studied in some other host.

While the life cycle of the coccidia is complex, it is at the same time simple and easily understood. The most confusion which comes to beginners is on account of the names, difficult to a neophyte, which are given to the different stages of the organism. These names, of course, have definite meanings to those familiar with them but to the uninitiated they are bizarre and difficult.

While the author is not unfamiliar with coccidia in certain other animals and has studied the species *Isospora belli,* through the courtesy of others, the rarity of coccidiosis of man in this country has made it impossible to give any material personal observations, and the discussion here is largely compiled from the writings of others, particularly Dobell and O'Connor (1921) and Wenyon (1926).

Description by word of the life cycle of coccidia usually makes a confusing thing out of what is really a simple process. It is best, therefore, to follow the custom of outlining the sequence of stages by diagram.

It is essential to an understanding of it to realize that the usual course of life is asexual but that periodically certain of the young organisms become differentiated into males and females and these conjugate to give a sexual cycle, presumably rejuvenating the race.

In reference to the accompanying diagram, which represents both cycles as they occur in a typical species which inhabits the epithelium of the intestinal mucosa, a graphic description of the scheme will be attempted in as simple terms as possible.

Starting with (1), the young coccidium, a minute ovoid organism within an epithelial cell, we see the parasite grow to a size where it practically occupies the whole housing cell, the cytoplasm of this cell being fed upon and the nucleus crowded and flattened to the bottom. This large asexual individual is called a Schizont (2). As it grows larger its nucleus divides and by repeated division becomes of a number characteristic of the species (3). The cytoplasm then splits (4) into as many new individuals as there are nuclei, this process of division being known by the term Schizogony. These young offspring are elongated cycle-shaped or wormlike bodies, called Merozoites. They are actively motile and their housing cell is burst (5) leaving the remnants of it and the parent Schizont. These Merozoites wiggle actively about and penetrate other host epithelial cells (6), in which they become rounded and from which point the asexual process of maturition

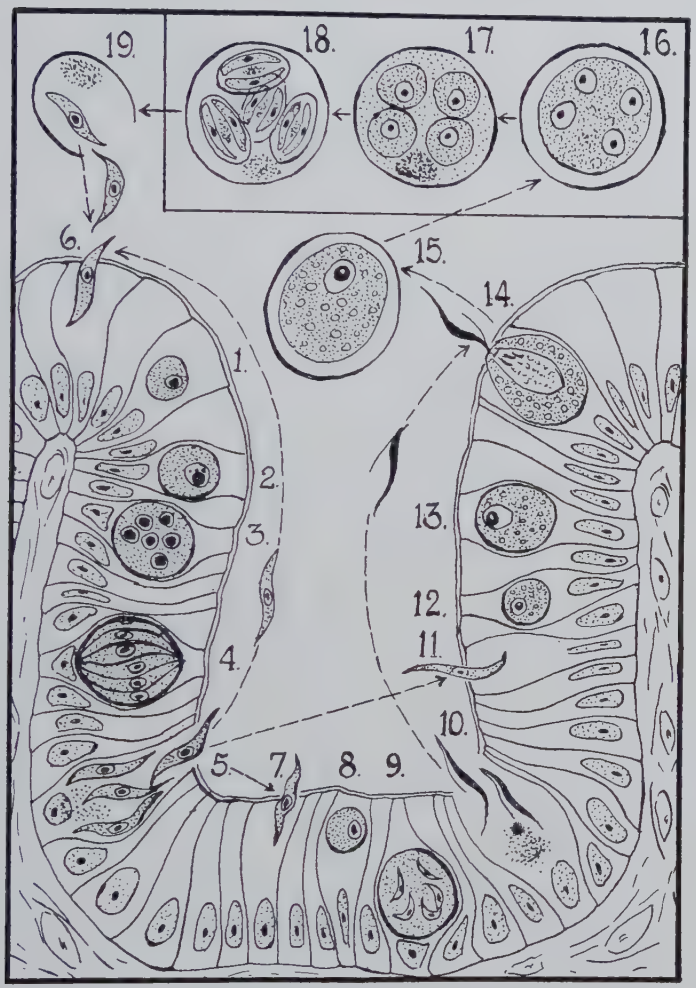


Fig. 35.—Life Cycle of Coccidia.

Diagrammatic representation of the asexual and the sexual cycles within intestinal epithelium and of the development of the cyst, stages 15–18, outside the body.

and division is repeated. This is the ordinary process taking place entirely within the parasite's habitat in the single host.

Periodically in the life of the parasite, supposedly as a rejuvenation process inherent in the organism, but due to unknown influences, the Merozoites, after penetrating epithelial cells, instead of maturing into asexual Schizonts, develop into male organisms, called Microgametocytes (7 and 8), and females (11), called Macrogametocytes. In the males the organism grows, at the expense of the host cell. the nucleus multiplies (9) and the body of the male splits off a number of flagellated individuals (10) corresponding to the number of multiplied nuclei. These bodies, called Microgametes, burst the confines of the host cell, leaving the remnant of it and the parent Microgametocyte and swim actively about until they come in contact with the female cell, corresponding to spermatozoa and their means of making contract with the ovum.

The young female (Macrogametocyte) after entering an epithelial cell does not multiply in a comparable fashion but enlarges and matures, at the expense of the cell host, into a single cell, called the Macrogamete (12, 13), thus corresponding to the ovum of the multicellular animal. The mature female element, the Macrogamete, is found by the male element, the Microgamete, within the remnant of the used up host cell (14). When the free swimming spermatozoon-like Microgamete, which is produced

in multiples of the female, thus continuing the analogy with germ cells of multicellular animals, meets the passive, large, ovum-like Macrogamete, it penetrates at one pole, the nuclei of the two unite, and the thus fertilized cell becomes the so-called Zygote (15), which is then an inert body in the lumen of the intestine.

Since this is the phase which is destined to continue the species in a new host and must withstand external exposure in the process of transfer, it must be resistant. It therefore becomes encysted, secreting a cyst wall, and is now called an Oöcyst. It is this cyst in some stage of development which is observed in the evacuated intestinal contents and upon which the diagnosis of the infection depends.

As the Oöcyst develops, passing down the intestine and even after passage in the stool, the protoplasm contracts and the nucleus divides and multiplies to a number characteristic of the species (16). Division of cytoplasm now occurs around the nuclei, to form an equal number of rounded bodies, called the Sporoblasts (17), leaving a remnant of the old protoplasm as unused residue. Each Sporoblast now secretes its own cyst wall, becoming encysted within the Oöcyst, the subcyst called the Sporocyst and each enclosed body now a Spore (18). The organism in each Spore divides into an equal and characteristic number of worm-like bodies, being called Sporozoites. This process of formation of spores in the Oöcyst is called Sporogony. The ripe Oöcyst (18) is

swallowed by a host-to-be, the Oöcyst and Sporocyst dissolved or burst (19) in the intestine, the Sporozoite (6), liberated and active, enters an epithelial cell and the process of asexual multiplication is begun in the new host.

The transmissible form of the organism, the Spore, is thus seen to be doubly protected, by the Oöcyst and the Sporocyst, the whole body resisting exposure and remaining viable under conditions more severe than may be borne by the comparatively non-resistant cysts of other intestinal protozoa.

HABITAT AND EFFECTS

Although it is not definitely known it is presumed that the coccidia of man inhabit the small intestine, although an infection of the liver has been reported.

The organisms live during their asexual life in the epithelium of the mucosa, consequently they are essentially pathogenic. The cells which are inhabited are destroyed as the organisms mature and become free. Naturally so long as this is all that occurs and unless there is sufficient denuding to expose the subepithelial tissues to bacterial invasion, it is not anticipated that any considerable reaction, functional disturbance, or symptoms, would occur from it. The loss and replacement of individual cells on the surface of the intestine is probably of constant occurrence under natural conditions. As a matter of fact there is no definite clinical state associated with

coccidiosis in man, and the host is usually, apparently, in good health. In the intestine of infected animals there is little evidence of reaction to the presence of the parasite. On the other hand severe and fatal diarrhoea or dysentery sometimes occurs in other animals. In the liver of the rabbit, which is commonly infected by its own species, there occurs a papilliferous bile duct lining proliferation, of tumor-like proportions at times. Wenyon (1926) notes a case, reported by Connal, who, having accidentally swallowed oöcysts of *I. belli,* developed diarrhoea and abdominal discomfort in six days. This persisted for four weeks and oöcysts appeared in the stool three weeks after the onset of symptoms, being present for twelve days. The stools became normal and recovery was complete.

In man intestinal coccidiosis is apparently a rare and a self-limited infection. Most of the cases reported have been in persons who had been in eastern Mediterranean countries. However, Kofoid, Kornhauser and Plate (1919), and Kofoid and Swezy (1920) reported finding eleven cases of the infection in United States troops, seven of whom had seen foreign service and four of whom had not. Haughwout (1921) reports a case from Manila which he thinks originated in the United States.

Other cases have been reported from West Africa, Java, Portuguese East Africa, Durban, Malay, China, Calcutta, Argentine, Brazil, and Saigon.

Altogether there have been some one hundred and

fifty cases put on record since Woodcock and Wenyon initiated the modern interest in the infection, in 1915.

The most recent light on the nature of the species occurring in man may be had from Wenyon (1926). According to this authority intestinal coccidiosis of man, in so far as it is known at the present, is due to the species named by him *Isospora belli.*

ISOSPORA BELLI

Woodcock (1915), according to Wenyon (1926), first discovered the immature oöcyst of this coccidium in feces of English soldiers invalided from Gallipoli. Wenyon (1923) observed the complete development of the oöcyst, proving it of the genus *Isospora,* and named it *Isospora belli.* According to him the coccidium found in some one hundred and fifty cases of intestinal infection since his and Woodcock's discoveries belong to this species. They have usually been identified as *Isospora hominis.*

The organism has been seen only in the oöcyst stage. It measures 25 to 33 microns in length and about half that in width. It is elongated, one end being constricted to the appearance of a neck.

The wall is of a double contoured outer layer and a thin inner membrane. Within this cyst is a rounded body of globules of refractive material, within a clear space, the size of this body and that of the surrounding space varying with the age of the cyst, the older the smaller the body of the enclosed

organism. Sometimes the first division, into sporoblasts, has occurred in fresh feces and there are two smaller enclosed bodies instead of one. Further development, the formation of sporocysts by the two sporoblasts, the division of each sporoblast into four sporozoites, leaving the rounded granular residual body in each sporocyst, has been induced and de-

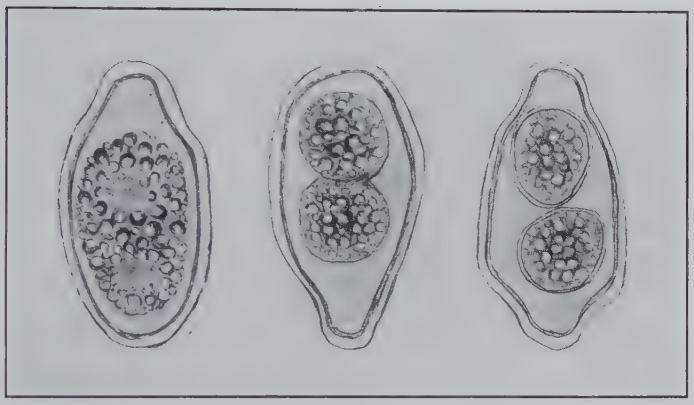


FIG. 36.—*Isospora belli.*

Drawings of oöcysts from human stool. x 1200.

scribed by Wenyon and has been observed by others to occur as the stool ages. The sporozoites are elongated comma shaped bodies, the cytoplasm granular, with a refractive globule in the larger end, the nucleus anteriorly but indistinct.

In feces these oöcysts are to be studied in wet unstained slide preparations. Since they are large they are readily picked up with the ordinary low power working lens. As they are transparent they may be

overlooked unless the lighting is properly adjusted. It is not improbable that they have occurred in feces which have been examined microscopically but have been overlooked. The impenetrability of the cyst makes staining unsatisfactory. In fresh feces it is only the first one or two stages of the oöcyst that occur. Stools aged at ordinary temperature develop in from one to three or four days, according to the climate. The number of cysts is small, as a rule, and they persist for only a few days, although Wenyon and O'Connor (1917) observed them during twenty-five days in one case.

Attempts to infect other animals experimentally have apparently been failures and literally nothing of proven nature, except the oöcysts, is known of the organism, its habitat or effects.

It is reasonably presumed that its habitat is the small intestine, where it passes the phase of schizogony in the epithelial cells, and that it is transferred from host to new host by the ingestion of oöcysts in fecal contaminated food and drink. Direct transfer, as is most effective in some other intestinal protozoa, seems unlikely, since the most favorable state would be the maturing of the oöcyst, with the formation of merozoites, before being swallowed by the host-to-be.

ISOSPORA HOMINIS

The infection of the interior of the villi of the mucosa of the small intestine of a man recorded by Virchow (1860) is, according to Wenyon, the only

reported instance of the occurrence of this species. This conclusion is based upon the preciseness of Virchow's record which would necessitate the organism being much smaller than that seen since 1915 and named by Wenyon *Isospora belli.*

Wenyon is inclined to think that *Isospora hominis* will be rediscovered upon careful examination of the mucosa of the small intestine.

It seems that *Isospora hominis* is like the small form of *Isospora bigemina* of cats and dogs and that it probably spends its life in the subepithelial tissues, where the oöcysts reach maturity.

The oöcyst, in that event, should be about one-half, or less, the size of *Isospora belli.* It should produce two sporoblasts and sporocysts, each with four sporozoites and residual body, in the matured stage. If otherwise comparable to the small form of *Isospora bigemina* in cats and dogs, its immature oöcysts are not released into the lumen of the intestine ordinarily, and so examination of feces would usually fail to reveal the presence of the infection. Since it should mature in the interstitial tissue of the villi, the oöcyst would be liberated only on disintegration of a diseased villus, and in mature form with two sporocysts containing four sporozoites and residual body each.

This is virtually a challenge to pathologists doing postmortem examinations to discover this infection by more careful microscopic section of the small intestine or by scrapings. That is, unless Virchow saw an accidental infection of man by *I. bigemina* from

the cat or dog, or unless he was in error in saying that it was like this small coccidium, with which he was, by the way, familiar.

EIMERIA GUBLERI

Since Thomson and Robertson (1926) have shown that the three supposed human intestinal species of the genus of coccidia known as *Eimeria, E. wenyoni, E. oxyspora,* and *E. snijdersi* (Dobell, 1919 and 1921), are merely species from fish, ingested and passing through the intestine in oöcyst stage, it seems that man's intestine is free of infection by this genus, although it is a common one in certain other animals.

The only instances of *Eimeria* infection of man remaining are five liver infections, according to Dobell (1921).

Gubler made the first report of such an infection in man in 1858 and, although it is not definitely proven that these cases were not really infections with *Eimeria stiedae,* a common coccidium of the liver of the rabbit, the name of Gubler has been given to this supposed species of man.

The oöcyst of this coccidium of man's liver is the only stage described and it only in immature form. It is of oval shape and about twenty microns in length.

If it is in reality *Eimeria steidae* of the rabbit or if it may be compared with that species, it should occur in the epithelial cells of the bile ducts in the

liver. There adenomatous tumors with papilliferous growth of the tubule linings occur in the rabbit infection. In the epithelium of these tumor-like bile duct growths may be seen the various stages of the endogenous cycle of development of the organisms. These tumorous foci of coccidial habitation produce gross white nodules in the liver substance. There is a chronic cellular reaction and increasing fibrosis associated with the papilliferous adenoma-like formation. The oöcysts escape from the bile ducts into the intestine and are passed in the feces, from which dissemination of the infection takes place. They are liberated from the epithelium and escape from the body in immature form. The oöcyst of *E. steidae* is from 20 to 40 microns in length and 16 to 25 microns wide. It is ovoid in shape, is of yellowish color, and one pole is flattened. The ripe oöcyst of the genus *Eimeria* is spherical and it has four spaces with two sporozoites each.

Further information as to the identity of the coccidium of man's liver is necessary before it can be finally placed. Certainly such a disease as occurs in the liver of the rabbit is not likely to be overlooked at autopsy. It must be rare to the degree of a pathologic curiosity.

CHAPTER XIV

BLASTOCYSTIS

Although the organisms known as *Blastocystis* are considered to be vegetable in nature and not protozoa, the confusion which has occurred in the past and is likely to occur in inexperienced hands at the present between these cells and protozoa makes it appropriate to include here a brief consideration of these parasites.

Alexeieff (1911) first called attention to the real nature of certain cells which had been previously described as cysts of *Trichomonas hominis*. He classified them as blastomycetes and gave the name *Blastocystis enterocola*.

Brumpt (1912), who gave the name *Blastocystis hominis* to the organism occurring in man, and Wenyon (1915), agreed with Alexeieff as to the nature of the organism. The writer (1917) reported it as a common intestinal parasite in this country, occurring in all of twenty-five cases of pellagra at autopsy and in the stools of 10% to 55% of several groups of individuals, mainly pellagrins. Barret (1921) first reported its cultivation, in human blood serum diluted ten times with 0.5% sodium chloride solution. Later

(Lynch, 1922A and 1922B), the writer reported upon culture experimentation with the organism and named two new species, *B. gemmagina* and *B. sporogina*, the former differing from *B. hominis* Brumpt in exhibiting peripheral budding in culture, and the latter in showing by the same method a process taken to be endosporulation. Since that time much incidental note has been taken of *Blastocystis*, particularly since it commonly grows profusely in a number of the culture mediums used for growing protozoa, often to the detriment of the purpose of the cultures.

Blastocystis is a very common body to be seen in the examination of feces. In the ordinary stool it is usually not numerous, is about 5 to 15 microns in diameter, usually rounded, but may be ovoid or more elongated. It has a delicate capsule, allowing alteration in shape. There is a sharp differentiation into peripheral and central zones, the former usually of uniform width in the rounded organisms and deeper at the poles of the ovoid or elongated than it is around the middle. This peripheral zone is transparent and colorless at times, at others greenish and refractive. In it occurs the nucleus or nuclei, appearing as highly refractive bodies.

The central body is rounded or ovoid, depending on the shape of the organism, and may be greenish and refractive, when the peripheral zone is transparent, or transparent, when the periphery is refractive. Not infrequently the division of the cell into

two, four or more, may be seen in the feces, and occasionally the peripheral budding.

In the stools of diarrhoea or dysentery, particularly in liquid stools with inflammatory exudate, and not uncommonly in the purged stool, the organism may

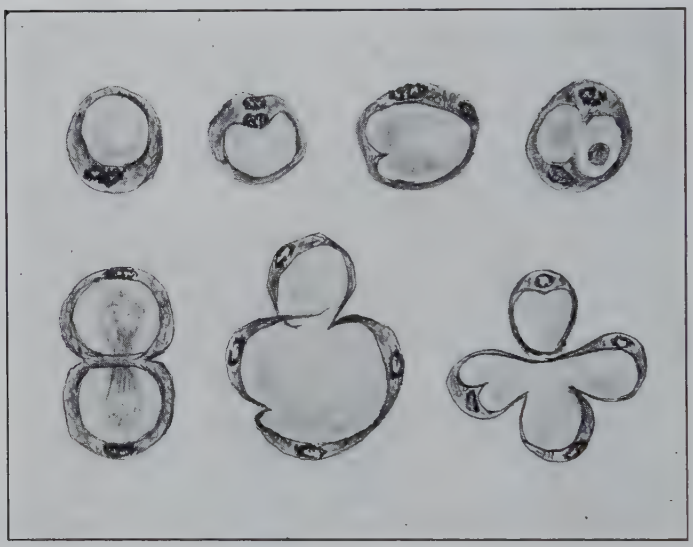


FIG. 37.—*Blastocystis hominis.*

Drawings of specimens from culture exhibiting the single cell and multiplication to produce two and four. Stained by iron-haematoxylon. x 900.

occur in enormous numbers and grow to large size, a diameter of 25 to 40 microns being not uncommon. In such specimens evidence of division is more common. In culture it grows profusely in several mediums, the serum saline medium being the simplest, attains large sizes and shows numerous dividing

forms. Young large forms in stool or culture are delicate of structure.

Division of the body in the stool is ordinarily by binary fission, the nucleus dividing apparently by mitosis and the two daughter nuclei becoming located at opposite poles. There is then a constriction of the body, both peripheral zone and central body, equally, to form symmetrical cells, or there may be multiple fission, with 2, 4, 6, 8, or more, similar daughter cells, all within a mucoid envelope which is apparently a product of the organism. Peripheral budding and endosporulation of species named *B. gemmagina* and *B. sporogina*, in addition to binary fission, may be studied in cultures.

Blastocystis is widespread in nature as a parasite of the intestine of many animals. It is not known as a free living body and is probably transmitted from person to person and from animal to animal through the contamination of ingested materials by fecal matter. It is an inhabitant of the colon in man. It is favored by inflammatory states of the intestine and from time to time it has been indicted, although not very seriously, as playing a causal rôle in intestinal disease. The writer (1923) recorded its presence in rectal ulcers, where it increased in size and numbers over those in the feces of the host, and exhibited active division.

The organism is readily found in examination of fresh wet preparations and, while it may be confused with protozoan cysts by the inexperienced, its char-

acteristic structure, once recognized, makes it easily differentiated and identified.

In thin smear preparations it is well stained by ordinary blood film methods, the peripheral zone, nuclei, and central body being differentiated. In iron-haematoxylon preparations the central body is dark, the peripheral zone lighter and the nuclei deeply stained.

In various of the mediums used for culture of intestinal protozoa it often grows when it has not been seen in the feces.

Although *Blastocystis* is an intestinal inhabitant of most or all people and has been seen very commonly in stool examination and culture we really know very little about its life. Its main importance at the present time lies in the need for its recognition by medical and other laboratorians.

REFERENCES

Aguilar, R. The Treatment of *Balantidium coli.* Ann. Report, United Fruit Co.; 14; 246; 1925.

Alexeieff, A. Sur la nature des formations dites "Kystes de *Trichomonas intestinalis.*" Comp. Rend, de la Soc. de Biol., 71; 296; 1911.

Allen, E. A. Encystment of *Councilmania lafleuri* in vitro. Univ. of Cal. Publ. Zool., 29; 175; 1926.

Baetjer, W. A. and Sellards, A. W. The behavior of amoebic dysentery in lower animals and its bearing upon the interpretation of the clinical symptoms of the disease in Man. Bull. Johns Hopkins Hosp., 25; 237; 1914.

Barret, H. P. A method for the cultivation of *Blastocystis.* Ann. Trop. Med. & Parasit., 15; 113; 1921.

Barret, H. P. and Yarborough, N. A method for the cultivation of *Balantidium coli.* Amer. Jour. Trop. Med., 1; 161; 1921.

Barret, H. P. and Smith, N. M. The cultivation of an *Endamoeba* from the turtle. Am. Jour. Hyg. 4; 155; 1924.

Barret, H. P. and Smith, N. Cultivation of *Endamoeba ranarum.* Ann. Trop. Med. & Parasit., 20; 85; 1926.

Barrett, M. T. The protozoa of the mouth in relation to pyorrhoea alveolaris. Dental Cosmos, 56; 948; 1914.

Bass, C. C. and Johns, F. M. New Orleans Med. and Surg. Jour., 67; 456; 1914.

Bass, C. C. and Johns, F. M. Pyorrhoea alveolaris and dentalis. Specific cause and treatment. Jour. Amer. Med. Assoc., 64; 553; 1915.

Bass, C. C. and Johns, F. M. *Alveolodental pyorrhoea.* Philadelphia. 1915.

Barrow, J. V. A clinical study of intestinal protozoa. Amer. Jour. Trop. Med., 4; 23; 1924.

Boeck, W. C. On the longevity of human intestinal protozoon cysts. Amer. Jour. Hyg., 1; 527; 1921.

Boeck, W. C. *Chilomastix mesnili* and a method for its culture. Jour. Exp. Med., 33; 147; 1921.

Boeck, W. C. and Stiles, C. W. Studies on various intestinal parasites (especially amoebae) of man. U. S. Hyg. Lab. Bull., No. 133; 1923.

Boeck, W. C. Studies on *Tricercomonas intestinalis.* Amer. Jour. Trop. Med., 4; 519; 1924.

Boeck, W. C. and Drbohlav, J. Cultivation of *Endamoeba histolytica.* Amer. Jour. Hyg., 5; 371; 1925.

Bensen, W. *Trichomonas intestinalis* und *vaginalis* des Menschen. Arch. Protist., 18; 115; 1910.

Blanchard, R. Remarques sur la megastome intestinal. Bull. Soc. Zool. France, 13; 18; 1888.

Brug, S. I. De *Entamoeben* van de rat. Geneesk. Tijd Nederl. — Indie., 59; 1; 1919.

Bohne, A., und Von Prowazek, S. Zur frage der flagellatendysenterie. Arch. Protist., 12; 1; 1908.

Boyd, M. F. A note on the cultivation of *Trichomonas intestinalis.* Journ. Parasit., 4; 168; 1918.

Brumpt, E. Demonstration du role pathogene du *Balantidium coli.* Enkystment et conjugaison de cit infusiore. Comp. Rend. Soc. Biol. Paris, 47; 103; 1909.

Brumpt, E. Colite a' *Tetramitus mesnili. Blastocystis hominis,* n. sp., et formes voisines. Bull Soc. Path. Exot., 5; 728; 1912.

Callender, G. R. Dysentery. Arch. Path. & Lab. Med., 3; 665; 1927.

Campbell, A. H. Med. Research Council, Spec. Rep. Series, No. 59, 1921.

Castex, M. R. and Greenway, C. J. *Chilomastix mesnili*, pathogenic role. Bull. Soc. Path. Exot., Feb. 11, 1925.

Chatterjee, G. C. On a five flagellated *Trichomonas* (n. sp.) parasitic in man. Indian Med. Gaz., 50; 1; 1915.

Chatton, E. Realisation experimentale chez le cobaye de l'amibiase intestinale a'*Entamoeba dysenteriae* Bull. Soc. Path. Exot., 10; 794; 1917.

Chiang, F. The rat as a possible carrier of the dysentery amoeba. Proc. Natl. Acad. Sci., 2; 239; 1925.

Chiavaro, A. Researches upon *Entamoeba histolytica.* Dental Rev., 28; 1122; 1914.

Cort, E. C. Amoebiasis of the liver. Jour. Amer. Med. Assoc., 90; 2005; 1928.

Councilman, W. T. and Lafleur, H. A. Amoebic dysentery. Johns Hopkins Hosp. Rep., 2; 395; 1891.

Craig, C. F. *Parasitic amoebae of man.* Philadelphia. 1911.

Craig, C. F. Observations on the cultivation of *Endamoeba histolytica.* Amer. Jour. Trop. Med., 6; 46; 1926.

Craig, C. F. *Parisitic protozoa of man.* Philadelphia. 1926.

Craig, C. F. Observations upon the hemolytic, cytolytic and complement binding properties of extracts of *Endamoeba histolytica.* Amer. Jour. Trop. Med., 7; 225; 1927.

Craig, C. F. Complement fixation in the diagnosis of infection with *Endamoeba histolytica.* Amer. Jour. Trop. Med., 8; 29; 1928.

Craig, C. F. Symptomatology, diagnosis and treatment

of carriers of *Endamoeba histolytica.* Jour. Amer. Med. Assoc., 90; 1347; 1928.

Craig, C. F. and St. John, J. H. The value of cultural methods in surveys for parasitic amoebae of man. Amer. Jour. Trop. Med., 7; 391; 1927.

Cutler, D. W. A method for the cultivation of *Entamoeba histolytica.* Jour. Path. & Bact., 22; 22; 1918.

Davis, C. H. and Colwell, C. *Trichomonas vaginalis* Donne. Jour. Amer. Med. Assoc., 92; 306; 1929.

Deeks, W. E. *Balantidium coli.* United Fruit Co. Rep., 14; 118; 1925.

Deeks, W. E. Amoebic dysentery. United Fruit Co. Rep., 17; 28; 1928.

Derrieu and Raynaud. Dysenterie chronique à flagellé nouveau. Bull. Soc. Path. Exot., 7; 571; 1914.

Dobell, C. *The amoebae living in Man.* London and New York. 1919.

Dobell, C. The discovery of the intestinal protozoa of Man. Proc. Roy. Soc. Med., 8; 1; 1920.

Dobell, C. and Jepps, M. W. A study of the diverse races of *Entamoeba histolytica* distinguishable from one another by the dimensions of their cysts. Parasit., 10; 320; 1918.

Dobell, C. and O'Connor, F. W. *The intestinal protozoa of Man.* New York. 1921.

Dock, G. Flagellate protozoa in the freshly passed urine of a man. Med. News, Dec. 22, 1894.

Drbohlav, J. J. Culture *d'Entamoeba gingivalis* (Gros 1849) Brumpt 1913. Ann. Parasit., 3; 361; 1925.

Escomel, E. Quelques remarques a propos des Trichomoniasis intestinale et vaginale. Bull. Soc. Path. Exot., 10; 553; 1917.

Escomel, E. El primer case de *Trichomonas* vesical descrite en el Peru. Rev. Med. Panamericana, 2; 61; 1927.

Evans, J. S. and Middleton, W. S. Jour. Amer. Med. Assoc., 64; 422, 1915.

Fantham, Stephens and Theobald. *Animal parasites of Man.* London and New York. 1916.

Faust, E. C. A new type of amoeba parasitic in Man observed in North China. Jour. Parasit., 9; 22; 1923.

da Fonseca, O. O. R. Sobre os flagellados das manuferas des Brazil. Um novo parasito do homen. Brazil. Med., 29; 281; 1915.

Glaessner, K. Ueber balantidienenteritis. Centralb. f. Bakt., 47; 351; 1908.

Goodey, T. A note on the trichomonad flagellate *Tetratrichomonas buccalis* n. sp. Parasitology, 9; 554; 1917.

Goodrich, H. P. and Moseley, M. On certain parasites in the mouth in cases of pyorrhoea. Jour. Roy. Micro. Soc., 513; 1916.

Gunn, H. *Councilmania lafleuri* not a new amoeba. Jour. Parasit., 9; 24; 1922.

Grassi, B. Dei. protozoiparasitti e specialmente de quelli che sono nell uomo. Gazz. Med. Ital. Lomb., 39; 445; 1879.

Grassi, B. Enterocolite conparasite. Nota preventiva. Gazz. Med. Ital. Lombardia, 39; 286; 1879.

Greene, J. L. and Scully, F. J. Diet in *Balantidium coli,* Jour. Amer. Med. Assoc., 81; 291; 1913.

Gros, C. Fragments d'helminthology et de physiologie microscopique. Bull. Cos. Imp. de Nat., de Moscow, 22; 549; 1849.

Hadley, P. B. The avenue and development of tissue infection in intestinal trichomoniasis. Bull. Rhode Island State Coll. Agr. Exp. Sta., 168; 1916.

Haughwout, F. G. Philippine Jour. Sc. (B), 8; 246; 1918.

Haughwout, F. G. A case of human coccidiosis detected in the Philippine Islands, with remarks on the devel-

opment and vitality of the cysts of *Isospora hominis* Rivolta. Philip. Jour. Sc., 18; 449; 1922.

Haughwout, F. G. The practical microscopic diagnosis of dysentery. Bull. Instit. Med. Res. Fe. Malay States. Manila. 1924.

Haughwout, F. G. Observations on the interpretation of the microscopic picture in dysentery and other intestinal disorders. Tr. Cong. Far East Assoc. Trop. Med., 1924.

Haughwout, F. G. and Callender, G. R. Dysentery: its diagnosis and management through the microscope. Internat. Clinics, 2; 1925.

Haughwout, F. G. and deLeon, W. On the ingestion of erythrocytes by *Pentatrichomonas* sp. found in a case of dysentery. Philippine Jour. Sc., 14; 207; 1919.

Haughwout, F. G., Domingo, E., and deLeon, W. Protozoologic and clinical studies on the treatment of protozoal dysentery with benzyl benzoate. Philip. Jour. Sc., 16; 633; 1920.

Hegner, R. W. The effects of changes in diet on the incidence, distribution and numbers of certain intestinal protozoa of rats. Amer. Jour. Hyg., 3; 180; 1923.

Hegner, R. W. Intestinal flagellates in Tropical America. Amer. Jour. Trop. Med., 5; 239; 1925.

Hegner, R. W. and Ratcliffe, H. Trichomonads in the mouth of the dog. Jour. Parasit., 14; 51; 1927.

Hegner, R. W. Transmission of intestinal protozoa from man and other animals to parasite-free fowls. Jour. Parasit., 15; 135; 1928.

Hegner, R. W. Experimental transmission of trichomonads from the intestine and vagina of monkeys to the vagina of monkeys. Jour. Parasit., 14; 261; 1928.

Hegner, R. W. Experimental studies on the viability and transmission of *Trichomonas hominis*. Amer. Jour. Hyg., 8; 16; 1928.

Hegner, R. W. The ingestion of red blood corpuscles by trichomonad flagellates. Jour. Amer. Med. Assoc., 90; 741; 1928.

Hegner, R. W. and Becker, E. R. The diagnosis of intestinal flagellates by culture methods. Jour. Parasit., 9; 15; 1922.

Hegner, R. W. and Payne, G. C. Surveys of the intestinal protozoa of man in health and disease. Sci. Monthly, 47; 1921.

Hegner, R. W. and Ratcliffe, H. Trichomonas from the vagina of the monkey. Jour. Parasit., 14; 27; 1927.

Hill, C. McD. The successful application of the culture method to the diagnosis of intestinal flagellates in the field. Amer. Jour. Hyg., 6; 646; 1926.

Hill, C. McD. and Hill, R. B. Infection with protozoa and the incidence of diarrhoea and dysentery in Porto Rico children of pre-school age. Amer. Jour. Hyg., 7; 134; 1927.

Hinshaw, H. C. On the morphology and mitosis of *Trichomonas buccalis*. Univ. Cal. Publ. Zool., 29; 159; 1926.

Hinshaw, H. C. Correlation of protozoa of human mouth with extent of certain lesions in pyorrhoea alveolaris. Proc. Soc. Exp. Biol. Med., 21; 71; 1926.

Hinshaw, H. C. Cultivation of *Trichomonas buccalis*. Univ. of Cal. Publ. Zool., 31; 31; 1927.

Hinshaw, H. C. Experimental infection of dogs with *Endamoeba gingivalis* and *Trichomonas buccalis* of human mouth. Proc. Soc. Exp. Biol. and Med., 25; 430; 1928.

Hlava. Dysentery in Bohemia. Abst. in Centralbl. f. Bact., 1; 537; 1887.

Hogue, M. J. The cultivation of *Trichomonas hominis*. Amer. Jour. Trop. Med., 1; 211; 1921.

Hogue, M. J. *Waskia intestinalis;* its cultivation and

cyst formation. Jour. Amer. Med. Assoc., 77; 112; 1921.

Hogue, M. J. Studies on *Trichomonas buccalis*. Amer. Jour. Trop. Med., 6; 75; 1926.

Hogue, M. J. *Trichomonas* in urine. Amer. Jour. Trop. Med., 7; 327; 1927.

Hogue, M. J. *Trichomonas* in tissue cultures. Amer. Jour. Trop. Med., 8; 325; 1928.

Hollander, E. *Giardia intestinalis* infection. Arch. Int. Med., 32; 522; 1923.

Howitt, B. F. The cultivation of *Endamoeba gingivalis* (Gros). Univ. of Cal. Publ. Zool., 28; 65; 1926.

Howitt, B. F. The effects of certain drugs and dyes upon the growth of *Endamoeba gingivalis* in vitro. Univ. of Cal. Publ. Zool., 28; 173; 1926.

James, W. M. Diagnosis of intestinal amoebiasis. Jour. Amer. Med. Assoc., 89; 1469; 1827.

James, W. M. Human amoebiasis due to infection with *Endamoeba histolytica*. Ann. Trop. Med. and Parasit., 22; 201; 1928.

James, W. M. and Deeks, W. E. The etiology, symptomatology and treatment of intestinal amoebiasis. Tr. Internat. Conf. on Health Problems in Trop. Amer. United Fruit Co., p. 271; 1924.

Jepps, M. W. Observations on intestinal protozoa in Malaya. Parasitology, 15; 213; 1923.

Jepps, M. W. and Dobell, C. *Dientamoeba fragilis*, n. g., n. sp., a new intestinal amoeba from Man. Parasit., 10; 352; 1918.

Jones, P. H. and Turner, R. H. Iodoxyquinoten sulphonic acid in the treatment of amebic dysentery. Jour. Amer. Med. Assoc., 93; 583; 1929.

Kartulis, S. Einiges uber die pathogenese der dysenterieamoben. Centralbl. f. Bakt., 9; 365; 1891.

Kessel, J. F. Experimental infection of rats and mice with

the common intestinal amoebae of man. Univ. of Cal. Publ. Zool., 20; 401; 1923.

Kessel, J. F. Intestinal protozoa of the domestic pig. Amer. Jour. Trop. Med., 8; 481; 1928.

Koch, D. A. An experimental study of the effects of dyes, of dye mixtures, and of disinfectants upon *Endamoeba gingivalis* (Gros) in vitro. Univ. of Cal. Publ. Zool., 29; 241; 1926.

Koch, D. A. Relation of moisture and temperature to the viability of *Endamoeba gingivalis* in vitro. Univ. of Cal. Publ. Zool., 31; 17; 1927.

Kofoid, C. A. Mitosis in *Trichomonas*. Proc. Nat. Acad. Sci's., 1; 315; 1915.

Kofoid, C. A. On the morphology and behavior of *Pentatrichomonas arden delteili* (Derrieu and Raynaud). Univ. of Cal. Publ. Zool., 20; 373; 1921.

Kofoid, C. A. *Karyamoeba falcata,* a new amoeba from the human intestinal tract. Univ. Cal. Publ. Zool., 26; 221; 1924.

Kofoid, C. A. *Karyamoebina* substituted for *Karyamoeba*, with a note of its occurrence in man. Univ. Cal. Publ. Zool., 26; 435; 1924.

Kofoid, C. A. Investigation on intestinal protozoa. Carnegie Institute Year Book, 1925.

Kofoid, C. A. *Councilmania tenuis* and *C. dissimilis,* intestinal amoebas of man. Arch. Int. Med., 41; 558; 1928.

Kofoid, C. A. Protozoa of the human mouth. Jour. Parasit., 15; 151; 1929.

Kofoid, C. A. and Allen, E. A. On the culture in vitro of *Councilmania lafleuri* and *Endamoeba coli.* Proc. Soc. Exp. Biol. and Med., 18; 310; 1921.

Kofoid, C. A., Boeck, W. C., Minnich, D. E. and Rogers, J. H. On the treatment of giardiasis in rats with arsenobenzol. Jour. Med. Research, 39; 293; 1919.

Kofoid, C. A., Kornhauser and Plate. Jour. Amer. Med. Assoc., 72; 1721; 1919.

Kofoid, C. A. and Swezy, O. On the prevalence of carriers of *Endamoeba dysenteriae* among soldiers returned from over-seas service. New Orleans Med. and Surg. Jour., 73; 4; 1920.

Kofoid, C. A. and Swezy, O. *Councilmania lafleuri,* a new amoeba of the human intestine. Proc. Soc. Exp. Biol. and Med., 18; 310; 1921.

Kofoid, C. A. and Swezy, O. On the morphology and mitosis of *Chilomastix mesnili.* Univ. Cal. Publ. Zool., 20; 117; 1921.

Kofoid, C. A. and Swezy, O. On the free, encysted and budding stages of *Councilmania lafleuri,* a parasitic amoeba of the human intestine. Univ. of Cal. Publ. Zool., 20; 169; 1921.

Kofoid, C. A. and Swezy, O. *Pentatrichomonas* in Man. Amer. Jour. Trop. Med., 4; 33; 1924.

Kofoid, C. A. and Swezy, O. The cytology of *Endamoeba gingivalis* (Gros) Brumpt compared with that of *Endamoeba dysenteriae,* with special reference to the determination of amoebas in bone marrow in arthritis deformans of Ely's second type. Univ. Cal. Publ. Zool., 26; 165; 1924.

Kofoid, C. A. and Wagener, E. H. Studies of the effect of certain drugs upon *Endamoeba dysenteriae* in vitro. Univ. of Cal. Publ. Zool., 28; 155; 1925.

Kofoid, C. A. and Wagener, E. H. A simplified medium for the cultivation of *Endamoeba dysenteriae.* Jour. Lab. and Clin. Med., 11; 683; 1926.

Kovacs, F. Beobachtungen und versuche uber die sogonnante amoebendysenterie. Ztschr. Heilk., 13; 509; 1892.

Kruse, W. and Pasquale, A. Untersuchengen uber dysenterie und leberabscess. Ztschr. f. Hyg., 16; 1; 1894.

Lambl, W. Mikroskopische untersuchengen der darmexkrete. Vierteljarsch. f. d. Prakt. Heidlk., 61; 1. Beobachtungen u. Studien aus. d. Gebiete d. pathol. Anat. u. Histol. aus. d. Franz-Joseph Kinderhospital Prag., 365.

Losch, F. Massenhafte entwickelung von amoben in Dickdarm. Arch. f. Path. Anat., 65, 196; 1875.

Lynch, K. M. Concerning endamoebiasis of the mouth. Amer. Jour. Trop. Dis. and Prev. Med., 3; 231; 1915.

Lynch, K. M. Trichomoniasis of the vagina and mouth. Amer. Jour. Trop. Dis. and Prev. Med., 2; 627; 1915.

Lynch, K. M. Clinical and experimental trichomoniasis of the intestine. N. Y. Med. Jour., May 1, 1915.

Lynch, K. M. The rat a carrier of a dysenteric amoeba. Jour. Amer. Med. Assoc., 65; 2232; 1915.

Lynch, K. M. Dauercystformation of *Trichomonas intestinalis*. Jour. Parasit., 3; 28; 1916.

Lynch, K. M. *Blastocystis hominis*, its characteristics and its prevalence in intestinal content and feces in South Carolina. Jour. Bact., 2; 369; 1917.

Lynch, K. M. Macrophages in feces in acute dysentery. Jour. Lab. and Clin Med., 4; 1917.

Lynch, K. M. Penetration of the intestine by *Endamoeba histolytica*. Jour. Amer. Med. Assoc., 75; 5; 1920.

Lynch, K. M. *Blastocystis* species in culture. Amer. Jour. Trop. Med., 2; 215; 1922.

Lynch, K. M. Cultivation of *Blastocystis* and determination of species. Amer. Jour. Trop. Med., 2; 539; 1922.

Lynch, K. M. A note on certain coprozoic organisms in culture from man. Amer. Jour. Trop. Med., 2; 223; 1922.

Lynch., K. M. Cutivation of *Trichomonas* from the human mouth, vagina and urine. Amer. Jour. Trop. Med., 2; 531; 1922.

Lynch, K. M. Cultivation of *Trichomonas,* and the question of differentiation of the flagellates. Jour. Amer. Med. Assoc., 79; 1130; 1922.

Lynch, K. M. *Tricercomonas intestinalis* and *Enteromonas caviae,* n. sp., and their growth in culture. Jour. Parasit., 9; 29; 1922.

Lynch, K. M. The occurrence of *Blastocystis* in intestinal inflammation, with a note on *Endolimax nana.* Jour. Amer. Med. Assoc., 81; 522; 1923.

Lynch, K. M. Notes on the natural and cultural growth of certain intestinal flagellates. Amer. Jour. Trop. Med., 4; 537; 1924.

Lynch, K. M. The ingestion of red blood corpuscles by an intestinal amoeba with eight nucleated cysts. Amer. Jour. Trop. Med., 4; 43; 1924.

Lynch, K. M. Intestinal flagellate infestation; a clinical analysis. Jour. Amer. Med. Assoc., 87; 4; 1926.

Lynch, K. M. The flagellated protozoa of the intestine. Amer. Jour. Trop. Med., 8; 345; 1928.

Lynch, K. M. The effects of intestinal protozoa. Proc. Inter-State Postgrad. Med. Assembly. 1928.

Lynch, K. M. Intestinal amoebiasis of the rat and man. Southern. Med. Jour., 21; 87; 1928.

Mackie, J. W. Ann. Trop. Med. and Parasit., 10; 291; 1916.

Manlove, C. H. Two cases of balantidial colitis. Philip-Jour. Sci., 12; 149; 1917.

Marchand, F. Uber das Vorkammen von *Trichomonas* im Harne eines Mannes, nebst Bermerkungen über *Trichomonas vaginalis.* Centralb. f. Bakt. u. Parasit., 15; 709; 1894.

Mason, C. W. A case of *Balantidium coli* dysentery, Jour. Parasit., 5; 137; 1919.

Matthews, J. R. and Smith. Med. Research Council., Spec. Rep. Series, No. 59, 1921.

Maxcy, K. F. *Giardia (Lamblia) intestinalis*, a common protozoan parasite of children. Johns Hopkins Hosp. Bull., 32, 1921.

Miura, R. *Trichomonas vaginalis* in frischgelassenen urin eines Mannes. Centralb. f. Bakt. u. Parasit., 16; 67; 1894.

Nutt, M. M. Med. Research Council, Spec. Rep. Series No. 59, 1921.

Ohira, T. and Noguchi, H. The cultivation of *Trichomonas* of the human mouth. Jour. Exp. Med., 25; 341; 1917.

von Prowazek, S. *Entamoeba buccalis* n. sp. Vorlanfige Miteilungen. Arb. Kais. Gesund., 21; 341; 1917.

Quincke, H. and Roos, E. Ueber amoeben enteritis. Berlin Klinische Wchnschr., 30; 1089; 1893.

Ratcliffe, H. L. The numbers of trichomonads in rats on diets of different protein content in relation to the pH and bacteria of the caecum. Amer. Jour. Hyg., 8; 910; 1928.

Rivas, D. The effect of temperature on protozoan and metazoan parasites and the application of intra-intestinal therapy in parasitic and other affections of the intestine. Amer. Jour. Trop. Med., 6; 47; 1926.

Smith, A. J., Middleton, W. S. and Barrett, M. T. The tonsils as a habitat of oral endamoebiasis. Jour. Amer. Med. Assoc., 63; 1746; 1914.

Smith, A. J. and Barrett, M. T. The parasite of oral endamoebiasis. Jour. Parasit., 1; 159; 1915.

Smithies, F. Protoziasis occurring in temperate zone residents. Amer. Jour. Trop. Med., 6; 1; 1926.

Steinberg, S. An investigation of the soft white matter which accumulates on the teeth and between them. Scoremenaya Meditsina. Kiev, 20; 377; 1862.

Steinberg, S. Kiewer. Ztschr. f. neucre Med. Quoted by Braun, M. Animal Parasites of Man. 1908.

Strong, R. P. The clinical and pathological significance of *Balantidium coli*. Pub. Bur. Govt. Labs. Manila, 26; 1; 1904.

Tanabe, M. Cultivation of trichomonads from man, rat and owl. Jour. Parasit., 12; 101; 1925.

St. John, J. H. Differential characteristics of amoebae from man in culture. Amer. Jour. Trop. Med., 6; 319; 1926.

Thomas, W. S. and Baumgartner, E. A. Stool examination for protozoa. Jour. Amer. Med. Assoc., 85; 1725; 1925.

Thompson, M. D. Experimental amoebiasis in the rabbit. Univ. Cal. Publ. Zool., 29; 9; 1926.

Thomson, J. G. and Robertson, A. Notes on the cultivation of certain amoebae and flagellates of man, using the technique of Boeck and Drbohlav. Jour. Trop. Med. and Parasit., 28; 345; 1925.

Thomson, J. G. and Robertson, A. Fish as the source of certain coccidia recently described as intestinal parasites of man. Brit. Med. Jour., 1; 282; 1926.

Ucke, A. Trichomonaden und Megastomum in Menschendarm. Centralb. Bakt. U. Parasit., 1. abt. 45; 231; 1908.

Wagener, E. H. A precipitin test in experimental amoebic dysentery in cats. Univ. Cal. Publ. Zool., 26; 15; 1924.

Wagener, E. H. and Thompson, M. D. Experimental amoebiasis in cats from acute and chronic human cases. Univ. of Cal. Publ. Zool., 26; 267; 1924.

Walker, E. L. A comparative study of the amoebae of the Manila water supply, in the intestinal tract of healthy persons and in amoebic dysentery. Philip. Jour. Med. Sci., 6; 259; 1911.

Walker, E. L. Experimental balantidiasis. Philippine Jour. Sci., 8; 333; Sec. B; 1913.

Walker, E. L. and Sellards, A. W. Experimental entamoebic dysentery. Philip. Jour. Sci., 8; 253; 1913.

Warsley, A. N. and Bateman, J. E. Urinary amoebiasis. Jour. Trop. Med. and Hyg., 27; 9; 1926.

Warthin, A. S. The occurrence of *Entamoeba histolytica* with tissue lesions in the testis and epididymis in chronic dysentery. Jour. Infec. Dis., 30; 559; 1922.

Wenyon, C. M. A new flagellate (*Macrostoma mesnili* n. sp.) from the human intestine, with some remarks on the supposed cysts of *Trichomonas*. Parasitology, 3; 210; 1910.

Wenyon, C. M. Experimental amoebic dysentery and liver abscess in cats. Jour. Lond. Sch. Trop. Med., 2; 27; 1912.

Wenyon, C. M. Observations on the common intestinal protozoa of man. Jour. Roy. Army Med. Corps, 25; 600; 1915.

Wenyon, C. M. The protozoological findings in 556 cases of intestinal disorder from the eastern Mediterranean war area. Jour. Roy. Med. Corps, 26; 445; 1916.

Wenyon, C. M. Histological observations on the possible pathogenicity of *Trichomonas intestinalis* and *Chilomastix mesnili*, with a note on *Endolimax nana*. Jour. Trop. Med. and Hyg., 23; 125; 1920.

Wenyon, C. M. On three supposed new *Entamoebae* of man; a review. Trop. Dis. Bull., 19; 182; 1922.

Wenyon, G. M. *Protozoology*. New York. 1926.

Wenyon, C. W. and O'Connor, F. W. *Human Intestinal protozoa in the Near East*. London. 1917.

Wight, T. H. and Prince, L. H. Artifacts in *Endamoeba* which have led to the naming of a new genus and species. Amer. Jour. Trop. Med. 7; 287; 1927.

Yorke, W. and Adams, A. R. D. Observations on *Entamoeba histolytica*. I. Development of cyst, ex-

cystation and development of excysted amoeba in vitro. Ann. Trop. Med. and Parasit., 20; 279; 1926.

Yorke, W. and Adams, A. R. D. Observations on *Entamoeba histolytica.* II. Longevity of cysts in vitro and their resistance to heat and to various drugs and chemicals. Ann. Trop. Med. and Parasit., 20; 317; 1926.

Zahorsky, J. Giardiasis in children. South. Med. Jour., 21; 595; 1928.

Zahorsky, J. and McLoon, M. Giardiasis in children. Jour. Amer. Med. Assoc., 88; 385; 1927.

INDEX OF AUTHORS

Macmillan 6-8-39